REVIEW ARTICLE

THE NITROGEN MUSTARDS

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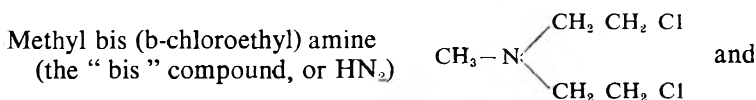
Introduction

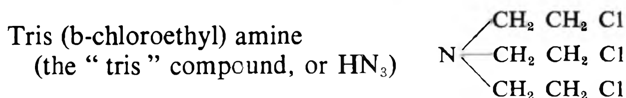
Mustard gas is well known for its local irritant action. It may also produce systemic disturbances if any is absorbed into the blood stream[1]. The nitrogen mustard derivatives were extensively investigated as potential chemical warfare agents in the recent war and particular attention was paid to their systemic effects. Animal studies showed that they inhibited the division of actively proliferating cells in the hæmatopoietic and lymphatic tissues. This action suggested that nitrogen mustard derivatives might be of value in the treatment of neoplastic conditions arising in the bone marrow or lymph glands. Clinical trials were started in the United States in 1943 and the first results collected from the various centres of investigation were published in 1946[2]. It was found that remission could be obtained in a number of neoplastic diseases. Unfortunately however serious toxic reactions were frequently encountered, because normal tissue is nearly as susceptible as tumour tissue to the action of the drug.

In the last five years widespread trials have made clear the therapeutic indications and the limitations of the original nitrogen mustard compounds. Research is now being directed to the synthesis and trial of new analogues. It is desirable to separate the toxic from the palliative action and if possible to find a curative agent.

The Chemistry of the Nitrogen Mustards

Original Derivatives. Gilman and Philips[3] in 1946 reviewed the chemistry of these compounds. The two simplest are

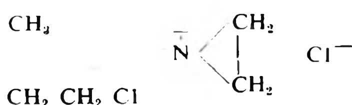

Methyl bis (b-chloroethyl) amine (the " bis " compound, or HN_2) $CH_3-N(CH_2CH_2Cl)_2$ and


Tris (b-chloroethyl) amine (the " tris " compound, or HN_3) $N(CH_2CH_2Cl)_3$

Most of the clinical trials have been conducted with the " bis " compound; it has a powerful tumour-inhibiting action and is the only nitrogen mustard commercially available in this country to-day. In August, 1950, the British Pharmacopœia Commission selected " Mustine " as the Approved Name for methyl bis (β-chloroethyl) amine, it is however marketed and used clinically as the hydrochloride not as the free base.

The "tris" compound has an equally powerful action, but has been largely discarded on account of its toxic effects, particularly a tendency to cause thrombosis on intravenous injection.

When these compounds are dissolved in water the molecule undergoes rearrangement, the nitrogen becomes pentavalent and a cyclic imonium compound is formed.



This imonium compound is very active and may become attached to chemical groups of body substances or be hydrolysed to an inactive chlorhydrin. The second β-chloroethyl group then undergoes rearrangement to form a much less active imonium compound and that finally hydrolyses. The formation and hydrolytic inactivation of the first imonium derivative proceeds very rapidly, the half life period of the "bis" compound in neutral solution is about 90 seconds at body temperature[4]. In practice the preparation used is the hydrochloride, and this gives an acid solution in water which is more stable[5]. On injection into a vein the solution is brought to the *p*H of blood plasma, but the high concentration of the chloride ion probably reduces imonium formation. When the molecule enters the cell, the chloride concentration is minimal and imonium ions are formed[6]. They react with amine, carboxyl or sulphydryl groups of the nucleus. An attempt to determine the site of imonium formation has been made with diethyl-iodoethylamine synthesised with radioactive iodine and given to mice. Comparison of the distribution of radioactivity with that following the injection of radioactive iodide suggested that the iodine was not being liberated from the nitrogen mustard derivative in the blood plasma[7].

New Compounds. The search for possible new therapeutic agents led first to the substitution of other halogens for chlorine in the original derivatives. No useful progress has been made in this direction[8]. The chemical grouping necessary for nitrogen mustard activity consists apparently of two chloroethyl groups attached to a nitrogen atom. It has however been shown that di-epoxides produce similar changes[9].

The basic *bis* chloroethylamine grouping can be modified in many ways with different aliphatic radicals and by the formation of double compounds. Burchenal[10] in the United States has been working on this problem and clinical trials have been made with a number of derivatives. 1:3-*bis* [*bis* (β-chloroethyl)] aminopropane dihydrochloride (SK 136) has been used for a number of patients[11,12,13,14]. It has been claimed to cause rather less nausea and vomiting than the "bis" compound but it may give rise to bad dreams, dizziness and headache. It does not appear to have any appreciable advantage over the original derivatives. Haddow[15] in this country has been studying aromatic substitution compounds. He has synthesised β-naphthyldi-2-chloroethylamine (R 48) which has possible therapeutic value. It is active when given by mouth although the action is slower and less powerful than the "bis" compound.

It does not produce the troublesome nausea and vomiting that so frequently follow the injections, but has the same action on the blood forming organs and may in addition cause a hæmorrhagic cystitis[16].

Screening Techniques. Several accounts have been published of the methods used for preliminary testing of new compounds in this series[4,10,17,18]. The first step is usually to determine whether the compound inhibits the development of leukæmia or sarcomata in mice. Compounds that are shown to have considerable activity are then tested for toxicity in animals and finally clinical therapeutic trials are initiated.

EXPERIMENTAL STUDIES OF THE ACTIONS OF THE NITROGEN MUSTARDS

The most striking changes seen in animals after the administration of the nitrogen mustards are in the nervous and reticulo-endothelial systems. Less marked changes occur in the gastro-intestinal tract the adrenals, testicles and skin. It has also been demonstrated that the mechanisms of blood coagulation and antibody production are affected. Very detailed studies have been made of the actions of these drugs on tissue cultures and mammalian cornea, but these will be considered in a subsequent section.

Neurological Effects. Large doses of the " bis " compound given to small animals produce neurological disturbances that are rapidly fatal. Parasympathomimetic, convulsant or paralytic phenomena may be observed[19], and histologically demyelination has been found in about one third of the animals[6]. The parasympathomimetic action is apparently related to the cyclization of the first chloroethyl group[20], whereas the paralytic action is produced by the methyl β-hydroxyethyl ethylenimonium derivative[20,21,22,23]. With the " bis " compound doses sufficient to produce neurological signs are invariably fatal, other nitrogen mustards however may give rise to permanent cerebellar or brain stem disturbances of movement[24].

Bone-marrow Effects. Mitotic division of the primitive white cells or red cells is inhibited, and these cells may largely disappear from the marrow[25,26]. Marrow culture studies have confirmed that the cells that fail to divide are actually killed[27]. The reticulum cells are apparently more resistant to the action of nitrogen mustard, but it has been suggested that they may be affected by the abnormal phagocytic activity required for the removal of the damaged cells[26]. If the aorta is occluded for 15 minutes after the injection, the marrow of the caudal part of the body escapes damage[28]. There is evidence in animal studies that if the red cell series is stimulated by the administration of phenylhydrazine the cells may be more resistant to subsequent nitrogen mustard action[29].

Spleen. Reduction occurs in the size of the spleen[19,25,26].

Lymphatic Glands. Necrosis of the germ centres has been observed within 5 hours of an injection of the " bis " compound and the lymphatic glands and thymus become smaller[19,25,26]. These changes are not mediated through the suprarenal cortical steroids as in the alarm reaction for they may still be demonstrated after adrenalectomy[28,30].

The Blood. There is a rapid fall in the number of circulating lymphocytes and a more gradual decline in granulocyte and red cell counts[19,25,26]. It has been suggested that the rapid disappearance of the lymphocytes may be due partly to their escape through the damaged gut wall[26].

Gastro-intestinal Tract. Vomiting and hæmorrhagic diarrhœa are seen in some animals and may be fatal through losses of fluid and electrolytes[5,19,31]. The vomiting is probably due to a local action on the gastric mucosa, but it has been suggested that it is partly central in origin[3]. The diarrhœa may be reduced by occluding the blood supply to the intestine for 15 minutes after the injection, but although the fluid loss is largely prevented, the animals may still die[31]. Histological changes have been demonstrated in the gastro-intestinal tract[19].

Adrenal Glands. Hypertrophy of the suprarenals with marked reduction of cortical cholesterol follows injections of the " bis " compound[32].

Liver. No change has been observed in normal liver cells, but regeneration of a rat's liver after partial hepatectomy is delayed by nitrogen mustard[33].

Kidney. No histological evidence of kidney damage has been found, but functional disturbances may aggravate the loss of fluid and electrolyte associated with gastrointestinal tract lesions[3].

Testicle. Temporary testicular damage has been demonstrated after large doses[34].

Hair. Subcutaneous injections in animals may lead to permanent greying of the hair at the site of the injection[35].

Antibody Production. The disappearance of the lymphocytes, unlike that following suprarenal cortical hormone, is not followed by an increase of circulating antibodies[36,37]. If the " bis " compound is given shortly before or at the same time as an antigen, production of antibodies is partially inhibited[36,37,38], and the development of the Schwarzman phenomenon may be prevented[39]. " Hypersensitivity " responses to foreign proteins, including renal lesions, are reduced by the administration of nitrogen mustard[40,41].

Complement. There has been one report of inhibition of complement[42].

Blood Coagulation. Prolonged clotting time and reduced heparin tolerance may follow injections of the " bis " compound. These disturbances may be corrected with intravenous heparin or toluidine blue[43].

Tumorigenic Action. The injection of the " bis " compound may be followed after a considerable interval by the development of a tumour. The neoplasm may appear at the injection site[44], in the lungs[45] or elsewhere[46].

Distribution in the body. Studies with radioactive elements incorporated in the molecule have shown that iodoethylamines are accumulated principally in the lungs, lymph glands and blood[7]. Injected " mustard gas " synthesised with radioactive sulphur is found in highest concentration in the lungs and kidneys. The bone marrow takes up comparatively little and the changes produced there are probably related to the greater sensitivity of the cells[47].

Cause of Death in Animals. Early deaths from large doses are associated with neurological disturbances. In some animals it may result from the loss of fluid and electrolyte through gastrointestinal changes. Damage to the blood-forming organs rarely leads to death in animal studies. It has been shown in animals that a dose of nitrogen mustard produces greater changes if given as a single injection as opposed to several small injections at short intervals[35].

Viruses, Bacteria, Protozoa. If influenza virus[48] or trypanosomes[49] are exposed to nitrogen mustard, their capacity for infecting mice is reduced. *Bact. coli* may acquire resistance to dilute solutions of the " bis " compound[50]. Concentrations of 500 mg./l. are viricidal and bactericidal in blood, and it is claimed that if the blood is then kept for 5 days it becomes non-toxic[51].

Mutation. Solutions of the " bis " compound produce mutations in *Penicillin notatum*[52] in *Drosophila*[53] and in strains of *Bact. coli*[50].

The Mechanism of Nitrogen Mustard Action

Animal studies have shown that the larger doses of nitrogen mustard act chiefly on the nervous system whereas with smaller doses changes are seen in tissues where there are actively proliferating cells. Research into the mechanism of these actions has proceeded along two main lines—the effect of dilute solutions on enzyme preparations, and observation of details of cellular changes produced in tissue cultures and intact tissues.

Enzyme Studies. Very dilute solutions of the " bis " compound inhibit the choline enzymes, oxidase, esterase, and acetylase. The neurological symptoms of nitrogen mustard poisoning may be produced in this way[54]. The inhibition may result from combination of the ethylenimonium ring with the SH groups of the enzymes[55], or alternatively the imonium ion, a quaternary ammonium derivative, may compete with choline for attachment to the enzyme[54]. Barron[54] has also reported the action of dilute solutions on the enzymes of carbohydrate metabolism. Pyruvate oxidase and phosphorylation enzymes are inhibited.

Tissue Preparations. Mitotic activity is brought to a standstill in cultures of embryonic tissue coming in contact with nitrogen mustard solutions[56,57,58]. In cultures of tumour tissue similar cytological changes have been demonstrated[57]. Friedenwald and his collaborators have made detailed studies of the changes produced in the mammalian cornea by nitrogen mustards[59,60]. Mitotic arrest and nuclear fragmentation have been found and also disturbances of the metabolism of the corneal stroma. Disturbances of metabolism have been demonstrated in other tissue preparations. Glycolysis is inhibited in human and rat skin[61], and oxygen consumption by slices of normal viscera[54] or of mouse sarcoma[62] is reduced.

Abnormalities of mitosis. The effect of the nitrogen mustards on cell proliferation is intimately related to abnormalities produced in mitotic division. Dustin[63] has reviewed the action of the various mitotic poisons and pointed out that the nitrogen mustards, like irradiation, lead to an inhibition of the prophase. This is followed by nuclear fragmentation

and death of the cell. It has, however, been observed that this nuclear fragmentation is more conspicuous after nitrogen mustard than after exposure to X-rays and it is possible that the actions are not identical[60]. The mitotic disturbances are probably due to damage to the chromosomes. It has been suggested that " chromosome stickyness " is increased[35]. They may be held together by attachment to the two chloroethyl groups[64] or as a result of a physical change in their thymonucleate coating[65,66]. The mitotic inhibition cannot be related to interference with nucleic acid phosphorylation[67].

Relation of Nitrogen Mustard Action to Irradiation

Nitrogen mustard and irradiation produce similar changes in the cell nucleus. Further comparisons have shown that the effects of the " bis " compound are produced more rapidly and are more severe, whereas those following exposure to deep X-rays develop slowly and are generally less intense. Recovery on the other hand starts earlier and is completed more quickly after injections of the " bis " compound[4]. Attempts to equate the effects produced in small animals suggest that a dose of 0·4 mg./kg. of the " bis " compound corresponds to overall irradiation of 140 r.[68] or 1·0 mg./kg. to 300 to 400 r.[21].

Clinical Applications

The results of animal studies suggested that the nitrogen mustards might produce a selective inhibition of actively proliferating tumours, particularly those originating in the blood-forming organs. The best results in clinical trials have been obtained in neoplastic conditions of hæmatopoietic and lymphatic tissues—the leukæmias and the lymphomata. The results in other neoplastic diseases have with one exception proved disappointing. The inhibition of experimental hypersensitivity phenomena in animals has led to an extension of the therapeutic trials to human disorders that are thought to be caused by comparable mechanisms. Unfortunately the practical value of the nitrogen mustards has been greatly reduced by their action on normal hæmatopoietic and gastro-intestinal tissues. The gap between therapeutic and toxic dose is virtually non-existent, anæmia, leucopenia and thrombocytopenia as well as troublesome nausea and vomiting follow the injections. The local irritant action which nitrogen mustard shares with the original sulphur mustard restricts the route of administration and may lead to additional complications.

Administration of Nitrogen Mustard. The hydrochloride of the "bis " compound is a white crystalline salt. It is available in 10 mg. bottles and immediately before injection should be dissolved in 10 ml. of sterile saline solution. If the solution is allowed to stand before being used some hydrolysis will occur. The " bis " compound must be administered intravenously. If injected straight into a vein with a syringe, thrombosis often follows. It is now usually injected into the tubing of a fast running intravenous saline drip infusion[69]. Veins partly occluded by tumour

should be avoided as they have a greater tendency to thrombose. If any of the solution escapes outside the vein severe inflammatory changes will be produced[70]. Solutions of the hydrochloride are not dangerous on the intact skin[21], but sensitisation dermatitis has been reported[71]. Intra-arterial administration has been tried in some cases[72] and intrapleural administration for patients with pleural effusion and multiple neoplastic deposits[73].

Dosage. The amount of nitrogen mustard that can be given as a single injection is limited by the nausea and vomiting produced. The amount that can be given in a course of injections over a short time is limited by the vulnerability of the bone marrow. It was originally suggested that a course should consist of four injections each of 0·1 mg./kg. of body weight on consecutive days[2]. The tendency now is to give fewer but larger doses [74], and up to 0·6 mg./kg. has been given as a single injection[75] and courses of as much as 3 mg./kg.[76]. In general, however, it is more satisfactory to adopt a conservative attitude and not to exceed 0·4 mg./kg. in each course and to limit the size of an individual dose to 0·2 mg./kg.[69].

Repetition of Courses. If two courses are given in rapid succession dangerous depression of the bone marrow may result. Provided the blood has returned to normal 4 weeks may be regarded as a safe interval[77].

"Prophylactic Therapy." If in any patient nitrogen mustard therapy is proving helpful, attempt may be made to keep the patient in prolonged remission by giving injections of 0·2 mg./kg. at regular intervals[69]. This is in contrast to the more usual procedure of awaiting a relapse before repeating the course of treatment. It has been suggested that the side reactions may be more severe after these " maintenance injections " [78].

Use in conjunction with X-ray therapy. Experimental studies in animals have shown that if irradiation is used before the " bis " compound the effects are not summated, whereas if the " bis " compound is given first more intense changes may be produced[4]. Attempts to combine the two forms of treatment have not led to spectacular results[73,78], and may be dangerous because of the difficulty of assessing the effects on the bone marrow.

Combination with Urethane treatment. Animal experiments have suggested that giving urethane with nitrogen mustard leads to increased action on lymphatic and neoplastic tissues[30,79]. Only one clinical trial of combined therapy has been reported and the details given are too meagre for critical evaluation[80].

Therapeutic Indications

Before nitrogen mustard therapy is recommended for any disease, a number of factors must be taken into account. It is not sufficient for the treatment to produce temporary reduction in the size of the tumour. Improvement in the patient's general condition and relief of symptoms for a reasonable period are criteria of satisfactory palliation[77,81]. The

discomforts and dangers of the toxic reactions must be considered and finally the results must be compared with those to be expected from alternative methods of treatment. Rhoads has summarised 1,100 reports of the use of the "bis" compound in the collective American trials and draws some, perhaps rather optimistic, conclusions concerning the indications for nitrogen mustard therapy[2,82].

Biopsy studies in a number of cases have confirmed the startling changes produced by this treatment in some neoplastic diseases[78,83,84,85,86]. There are several reports of autopsies carried out shortly after a course of the "bis" compound, in which little or no remaining neoplastic tissue could be found. The cause of death presumably being either the toxic action of the drug or previous disorganization of the patient's tissues produced by the neoplasm[87,88,89].

The alternative treatment for most of the diseases in which the "bis" compound has been tried is deep X-ray therapy. In assessing the value of the new agent, it is therefore necessary to attempt to compare the results obtained by the two methods. The suggestion is often made that nitrogen mustard therapy may be helpful when the neoplasm becomes "radio-resistant." This is an unsatisfactory term and it is seldom defined in case reports. It may mean that the tumour cells have ceased to respond to X-ray therapy, or alternatively that X-ray therapy has ceased to be practicable either because there are no tumours suitable for treatment or because the limit of skin tolerance has been reached[69]. Karnofsky has pointed out that the mechanism of action of the two agents is similar, and that if the tumour cells are resistant to irradiation, they will not respond to nitrogen mustard[68]. In the other types of so-called X-ray resistance the "bis" compound may produce a good remission.

The Lymphomata. Neoplastic conditions of the lymphatic system are grouped together under this heading. It has been divided into various subgroups in a number of systems of classifications. Pathologists unfortunately often differ in their interpretations of these classifications and it is simpler to consider only the well defined types of lymphomata.

Hodgkin's Disease. The best results of nitrogen mustard therapy have been obtained in this condition. In early cases with localised changes both X-rays and the "bis" compound will cause regression, that following irradiation lasts considerably longer, however, and therefore X-ray therapy is the treatment of choice. If the signs of the disease are widely disseminated and the patient is febrile, the "bis" compound is likely to lead to a more complete remission[2,74,78,90]. Certain manifestations usually respond well, for example skin lesions[70,91], paraplegia[92] or pulmonary infiltrations [93,94], whereas with generalised skin irritation or bone lesions the results are likely to be disappointing[70,90]. Large glandular masses causing obstructive symptoms may, with X-ray therapy, show initial enlargement before ultimately responding. Treatment with the "bis" compound does not produce any swelling and is therefore a safer method[91]. The remissions produced by nitrogen mustard vary in duration from a few days to a year or more. When the effects of the first course are beginning to disappear, provided the blood condition is satis-

factory, the treatment may be repeated. In some patients the symptoms have been kept in check for several years[81].

Lymphosarcoma. Results of nitrogen mustard therapy are less consistent, but if the disease is of the slowly progressing type satisfactory remission may be obtained[82,95].

Reticulosarcoma. This is the most rapidly fatal type of lymphoma. The tumours often respond dramatically to the "bis" compound, but the remissions are too transient to be of any value[95].

Giant Follicular Lymphoma. Although satisfactory remissions may be obtained the results are not so good as those following irradiation[90,95].

Mycosis Fungoides. In this condition the lymphomatous deposits are for the first few years confined to the skin. The results of therapy with the "bis" compound are very variable but quite good in some cases[89,95,96,97,98].

The Leukæmias. In acute leukæmias the "bis" compound is of no value. It may precipitate a fatal issue by damaging the remaining hæmatopoietic tissue. In chronic myeloid leukæmia useful remissions may be obtained[13,99], but if X-ray therapy is available, it is to be regarded as the treatment of choice. In chronic lymphatic leukæmia, the white blood cell count and lymph glands usually respond, but the general condition and any anæmia that may be present, rarely improve[13,77,99].

Multiple Myeloma. This condition shows little response to the "bis" compound although bone pains may be relieved for a time[100].

Polycythæmia Vera. Satisfactory remissions may be produced by the "bis" compound, but it is too early to compare the long term results with those following irradiation by deep X-ray or radioactive phosphorus[101]. Some clinicians regard nitrogen mustard therapy as dangerous in this condition[95].

Carcinoma. The results of therapeutic trials have with the exception of bronchial carcinoma proved disappointing[73,82]. Secondary deposits in the lungs may show slight, transient regression[102].

Primary Carcinoma of the Lung. Symptomatic remissions in cases of inoperable bronchial carcinoma follow nitrogen mustard therapy. Deep, but not pleural, pain and shortness of breath may be relieved, there is often improvement in the patient's general condition, and possibly on occasions some prolongation of life[35,73,76,102]. The results are comparable to those produced by palliative irradiation. It is not clear why bronchial growths should respond while others fail to do so. It has been suggested that these tumours occur in a crowded area where very slight reduction in size, that would pass unnoticed in less vital areas, may lead to considerable amelioration of symptoms[73]. Anaplastic tumours show most marked response but all histological types of bronchial neoplasm may be influenced by nitrogen mustard therapy.

Other Tumours. Patients with melanomata[82], primitive nerve cell tumours[75], testicular tumours[73], Kaposi sarcoid[89,98], eosinophil granuloma or Letterer Siwe disease[97] derive little benefit from nitrogen mustard.

Sarcoidosis. Several cases of this condition have been treated but no definite benefit can be claimed[103,104,105].

Skin Conditions. Nitrogen mustard therapy in the cutaneous manifestations of the lymphomata has already been mentioned. Relief of irritation in two cases of non-leukæmic dermatoses has been reported[95] and a satisfactory remission in a single case of disseminated lupus erythematosus[89]. Psoriasis is not influenced by it[97]. Aleksandrowicz considers that nitrogen mustard acts by stimulating reticuloendothelial activity, granulation tissue, and the occurrence of fibrosis. He claims to have treated neoplastic, decubitus and other chronic ulcers with considerable benefit[106,107,108].

Nephritis. In animals the "bis" compound inhibits hypersensitivity reactions. This has led to its trial in cases of human subacute nephritis but no definite benefit can yet be claimed[109].

Gastric Ulcer. The "bis" compound may inhibit the secretion of gastric acid. In a short series of cases of peptic ulcer treatment has been reported as being helpful[110]. The value of such therapy appears doubtful.

Tuberculosis. Aleksandrowicz, as a result of his unorthodox views on the action of the "bis" compound, has tried it for patients with chronic tuberculosis. He has claimed improvement in cases of spinal caries[111] and of cervical gland and pulmonary tuberculosis[108,112].

Toxic and Side Effects of Nitrogen Mustard Therapy

Venous Thrombosis. Thrombosis of the vein used for the injection of the "bis" compound has already been discussed. Transient local discomfort is usually produced and in one case a fatal pulmonary embolus has followed[113]. If many courses of the "bis" compound are given and widespread thrombosis occurs, blood transfusion, so often required as part of the treatment, may become very difficult.

Nausea and Vomiting. In the majority of patients troublesome nausea and vomiting begin about 2 hours after the injection and continue over the next 4 hours[35,78]. The severity of these reactions is to some extent proportional to the size of the dose, but may decrease after the later injections. Anorexia is usual during the course of treatment, but if the neoplasm has responded well, an excellent appetite soon returns. Various drugs have been tried to influence the nausea and vomiting. A barbiturate sedative is often employed but its value is doubtful. Pyridoxin, 100 mg. by intramuscular injection, has been given, again without any clear-cut benefit[70,78,95,102]. It should not be injected until 30 minutes after the "bis" compound as it may inactivate it. Benadryl and atropine are of no value[103]. Not only is the vomiting distressing to a seriously ill patient, but it may also be dangerous. Dehydration may be produced[90]. Serious hæmatemeses have been reported[78,95,114]. In two patients with thrombocytopenia, the vomiting has caused fatal cerebral hæmorrhage[90,95]. In a few patients nitrogen mustard treatment has been followed by the development of a peptic ulcer[95,115].

Diarrhœa. This has sometimes been noted, but is much less marked in patients than in animals[35,99].

Rigors. These have occasionally been described as complications of

nitrogen mustard therapy but are more likely to be related to the injection technique[35,78,88,100].

Bone Marrow. The changes observed in serial bone marrow punctures have been described by Spurr and his co-workers[83]. Depression of the red and white cell precursors is maximal in the second week and it takes several weeks for the marrow to return to normal. Recticulum cells and plasma cells are more resistant to the " bis " compound. Similar studies have been made in this country[116].

Lymphatic Glands. Serial biopsies have shown cytological changes with an apparent increase of fibrous tissue. The alterations in the lymphatic glands appear before those in the bone marrow[83,85].

Spleen. Splenic puncture shows reduction in the size of the sinusoids and often complete disappearance of the Malpighian corpuscles[83,85,86].

The Blood. Within a few hours of injection of the " bis " compound a lymphopenia develops accompanied by a transient increase in the granular cell count. This soon gives place to a neutropenia, maximal in the third week and recovering usually by the sixth. Reticulocytes become scanty soon after the injection and do not reappear until the third week. There is usually a slight fall in the red cell count but occasionally a sharp reduction occurs in the second week possibly as the result of hæmolysis. A few patients who are anæmic when the injections are given, and in whom a good response is obtained, may show a steady rise in the hæmoglobin level after treatment[69]. Thrombocytopenia is usually noticeable by the third week[70,116]. Various attempts have been made to protect the bone marrow from the action of nitrogen mustard. Choline and hexamine have been tried without any success[117,118]. Placing tourniquets round the limbs for 2 minutes after the injection of the " bis " compound has not altered the hæmatological sequelæ[73]. If the action on the bone marrow is unusually severe, agranulocytosis, severe anæmia or thrombocytopenic purpura may result.

Agranulocytosis. The development of this condition usually results from the administration of too much of the " bis " compound. In some patients and in some diseases, however, the bone marrow may be unusually sensitive. Patients in the terminal stages of lymphomatous diseases are more liable to develop hæmatological complications[88], whereas those suffering from bronchial carcinoma seem to be relatively resistant[35]. Pharyngitis[91], stomatitis[74,76] and lung infections may develop in the absence of the normal body defences. In some cases penicillin, with or without blood transfusion, has enabled the patient to recover from this complication, but it has not infrequenly proved fatal.

Extreme caution should be exercised in treating a patient with a leucopenia. Patients with lymphomata sometimes have very low white cell counts before any treatment has been given, and splenectomy has been advised to correct this prior to giving nitrogen mustard[119]. However, if the bone marrow can be shown to contain normal numbers of granulocyte precursors, the leucopenia may be disregarded, and a rise in the white cell count may be anticipated after the treatment[81].

Anæmia. It is difficult to dissociate the anæmia due to the disease from

that produced by the " bis " compound. The occasional sudden drop in red cell counts shortly after a course of injections has already been mentioned. This does not necessarily mean that treatment has failed, and if the patient is maintained by blood transfusion a remission may still follow[69].

Purpura. Although reduction of the platelet count is usual, purpuric eruptions are not very common[115,116]. Deaths from hæmorrhage have been reported[75] and these have been related to thrombocytopenia rather than to the defect of blood coagulation which has been observed[43]. The prolongation of the clotting time has been associated with the presence of a heparin-like substance in the plasma but others have been unable to confirm this finding[120].

Liver Damage. Histological studies in one series of patients dying shortly after nitrogen mustard therapy failed to reveal any specific changes in the liver[86]. There are, however, reports of liver cell necrosis[78] and of deaths from liver failure[75,97,103]. The latter may have been due to the underlying disease or homologous serum jaundice rather than the results of nitrogen mustard therapy.

Testicular Damage. A reduction of active spermatogenesis following the " bis " compound has been reported by several observers[85,86,88].

Neurological Disturbances. Although these were frequently observed in animal investigations, they are not common in man, probably on account of the relatively smaller dose used. Lightheadedness and drowsiness have been mentioned[35], toxic psychosis has been reported in one patient[121] and encephalopathy in another[75]. With some of the newer nitrogen mustards that have been used in more recent clinical trials, neurological symptoms have been quite common[12].

Skin Reactions. Maculo-papular rashes[115], a varicelliform eruption[88] and exfoliative dermatitis[103] have on rare occasions followed nitrogen mustard therapy. It has been claimed that the risk of general toxic reactions can be foretold by the result of a skin test with various dilutions of the " bis " compound[106,107]. It appears, however, that the results of this test depend entirely on the texture of the skin[118].

Other tissues. The suprarenals and kidneys do not show any specific changes in patients dying shortly after a course of the " bis " compound.

Balance Studies. Negative nitrogen and potassium balances have been demonstrated after nitrogen mustard administration[122]. Urinary coproporphyrin excretion is increased[123].

Plasma Proteins. Electrophoretic studies of plasma proteins after administration of the " bis " compound show no consistent change[118,124].

Conclusions

The nitrogen mustards, studied in the course of chemical warfare research, have proved a useful therapeutic agent in medicine. They are of value in the management of neoplastic conditions of the lymphatic tissues and blood-forming organs. Unfortunately, however, their action is only palliative, and unpleasant and dangerous side effects may be produced.

The original nitrogen mustards form the basis of numerous potential chemo-therapeutic agents and research is proceeding to find new compounds that are more active and less toxic. So far no new analogue has been found that is of greater value in practice than the original "bis" nitrogen mustard.

Since this article has been prepared, two extensive American reviews have arrived in this country. Philips (*Pharmacological Reviews* 1950, p. 281) discusses the pharmacological aspects, and Karnofsky (*Advances in Internal Medicine*, Vol. IV, Interscience Publishers London 1950, p. 1) covers a wider field.

References

1. Krumbhaar and Krumbhaar, *J. med. Res.*, 1919, **40,** 497.
2. Rhoads, *J. Amer. med. Ass.*, 1946, **131,** 656.
3. Gilman and Philips, *Science*, 1946, **103,** 409.
4. Karnofsky, Burchenal, Ormsbee, Cornman and Rhoads, *Approaches to Tumour Chemotherapy*, American Association Advancement of Science, 1947, 293.
5. Boyland, *Brit. J. Pharmacol.*, 1946, **1,** 247.
6. Anslow, Karnofsky, Jager and Smith, *J. Pharmacol.*, 1947, **91,** 224.
7. Seligman, Rutenberg and Friedman, *J. nat. Cancer Inst.*, 1949, **9,** 261.
8. Justin-Besançon, Lamotte-Barrillon and Polonovski, *Bull. Soc. méd. Hôp., Paris*, 1949, **65,** 29.
9. *27th Annual Report of the British Empire Cancer Campaign*, 1949, 33.
10. Burchenal, Lester, Riley and Rhoads, *Cancer*, 1948, **1,** 399.
11. Bethel, Andrews, Neligh and Myers, *Amer. J. Roentgenol.*, 1950, **64**, 61.
12. Burchenal, *Radiology*, 1948, **50,** 494.
13. Burchenal, Myers, Craver and Karnofsky, *Cancer*, 1949, **2,** 1.
14. Levin and Holt, *Texas St. J. Med.*, 1949, **45,** 551.
15. Haddow, Kon and Ross, *Nature*, 1948, **162,** 824.
16. Matthews, *Lancet*, 1950, **258,** 896.
17. Landing and Eisenberg, *Cancer*, 1949, **2,** 1083.
18. Shapiro, Goldin, Landing, Bergner, Faiman and Goldberg, *Cancer*, 1949, **2,** 100.
19. Graef, Karnofsky, Jager, Krichesky and Smith, *Amer. J. Path.*, 1948, **24,** 1.
20. Philips and Gilman, *Advances to Tumour Chemotherapy*, American Association Advancement of Science, 1947, 285.
21. Boyland, *Biochem. Symp.*, 1948, **2,** 61.
22. Foss, *Quart. J. exp. Physiol.*, 1947-8, **34,** 279.
23. Hunt and Philips, *J. Pharmacol.*, 1949, **95,** 131.
24. Goldin, Noe, Landing, Shapiro and Goldberg, *J. Pharmacol.*, 1948, **94,** 249.
25. Cameron, Courtice and Jones, *J. Path. Bact.*, 1947, **59,** 425.
26. Kindred, *Arch. Path.*, 1947, **43,** 253.
27. Osgood and Chu, *Cancer Res.*, 1950, **10,** 98.
28. Karnofsky, Graef and Smith, *Amer. J. Path.*, 1948, **24,** 275.
29. Jacobson, Marks, Gaston and Block, *J. Lab. clin. Med.*, 1949, **34,** 902.
30. Bass and Feigelson, *Cancer Res.*, 1948, **8,** 503.
31. Houck, Crawford and Bannon, *J. Pharmacol.*, 1947, **90,** 277.
32. Ludewig and Chanutin, *Endocrinology*, 1946, **38,** 376.
33. Landing, Seed and Banfield, *Cancer*, 1949, **2,** 1067.
34. Landing, Goldin and Noe, *Cancer*, 1949, **2,** 1075.
35. Boyland, Clegg, Koller, Rhoden and Warwick, *Brit. J. Cancer*, 1948, **2,** 17.
36. Philips, Hopkins and Freeman, *J. Immunol.*, 1947, **55,** 289.
37. Spurr, *Proc. Soc. exp. Biol., N.Y.*, 1947, **64,** 259.
38. Taliaferro and Taliaferro, *J. inf. Dis.*, 1948, **82,** 5.
39. Becker, *Proc. Soc. exp. Biol., N.Y.*, 1948, **69,** 247.
40. Bukantz, Dammin, Wilson, Johnson and Alexander, *Proc. Soc. exp. Biol., N.Y.*, 1949, **72,** 21.
41. Schwab, Moll, Hall, Brean, Kirk, Hawn and Janeway, *J. exp. Med.*, 1950, **91,** 505.

42. Watkins and Wormall, *Nature,* 1948, **162,** 535.
43. Jacobson, Marks, Gaston, Allen and Block, *J. Lab. clin. Med.*, 1948, **33**, 1566.
44. Boyland and Horning, *Brit. J. Cancer,* 1949, **3,** 118.
45. Heston, *J. nat. Cancer Inst.*, 1949, **10,** 125.
46. Griffin, Brandt and Tatum, *J. Amer. med. Ass.*, 1950, **144,** 571.
47. Dixon and Needham, *Nature,* 1946, **158,** 432.
48. Rose and Gellhorn, *Proc. Soc. exp. Biol., N.Y.*, 1947, **65,** 83.
49. Chen, *J. inf. Dis.*, 1948, **82,** 133.
50. Bryson, *J. Bact.*, 1948, **56,** 423.
51. Hartman, Mangun, Feeley and Jackson, *Proc. Soc. exp. Biol.. N.Y.*, 1949, **70**. 248.
52. Stahmann and Stauffer. *Science,* 1947, **106,** 35.
53. Auerbach, Robson and Carr, *Science,* 1947, **105,** 243.
54. Barron, Bartlett and Miller, *J. exp. Med.*, 1948, **87,** 489, 503.
55. Bain, *Amer. J. Physiol.*, 1950, **160,** 187.
56. Bodenstein, *Cancer Res.*, 1947, **7,** 49.
57. Cornman and Ormsbee, *Fed. Proc.*, 1947, **6,** 390.
58. Fell and Allsopp, *Cancer Res.*, 1949, **9,** 238.
59. *Bulletin Johns Hopkins Hospital,* 1948, **82,** (Feb.).
60. Friedenwald, Buschke, Scholz and Moses, *Approaches to tumour chemo therapy,* American Association Advancement of Science, 1947, 358.
61. Barron, Meyer and Miller, *J. invest. Derm.*, 1948, **11,** 97.
62. Jandorf, Calkins and Goldin, *Fed. Proc.*, 1947, **6,** 264.
63. Dustin, *Nature,* 1947, **159,** 794.
64. Ross, *Nature,* 1950, **165,** 808.
65. Gjessing and Chanutin, *Cancer Res.*, 1946, **6,** 593.
66. Gjessing and Chanutin, *Cancer Res.*, 1946, **6,** 599.
67. Friedenwald and Crowell, *Johns Hopk. Hosp. Bull.*, 1949, **84,** 568.
68. Karnofsky and Burchenal, *Amer. J. Med.*, 1950, **8,** 767.
69. Nabarro, *Brit. med. J.*, 1949, **2,** 622.
70. Karnofsky, Craver, Rhoads and Abels, *Approaches to Tumour Chemotherapy,* American Association Advancement of Science, 1947, 319.
71. Goldman, *J. Amer. med. Ass.*, 1947, **135,**, 1123 (discussion).
72. Bierman, Byron, Miller and Shimkin, *Amer. J. Med.*, 1950, **8,** 535.
73. Karnofsky, Abelman, Craver and Burchenal, *Cancer,* 1948, **1,** 634.
74. Ap Thomas and Cullumbine, *Lancet,* 1947, **252,** 899.
75. Bierman, Shimkin, Mettier, Weaver, Berry and Wise, *Calif. Med.*, 1949, **71**. 117.
76. Lynch, Ware and Gaensler, *Surgery,* 1950, **27,** 368.
77. Winkrobe and Huguley, *Cancer,* 1948, **1,** 357.
78. Damashek, Weisfuse and Stein, *Blood,* 1949, **4,** 338.
79. Skipper, *Cancer,* 1949, **2,** 475.
80. Merk, *Med. Klinik,* 1948, **22,** 629.
81. Spurr, Smith, Block and Jacobson, *Amer. J. Med.*, 1950, **8,** 710.
82. Rhoads, *Trans. Amer. Ass. Physicians,* 1947, **60,** 110.
83. Block, Spurr, Jacobson and Smith, *Amer. J. clin. Path.*, 1948, **18,** 671.
84. Cornell and Blauw, *Amer. J. Path.*, 1949, **25,** 233.
85. Gaensler, McKay, Ware and Lynch, *Arch. Path.*, 1948, **46,** 503.
86. Spitz, *Cancer,* 1948, **1,** 383.
87. Block and Murphy, *Arch. Path.*, 1948, **46,** 519.
88. Justin-Besançon, Lamotte-Barrillon and Polonovski, *Sem. Hôp., Paris,* 1948, **24,** 1518.
89. Osborne, Jordon, Hoak and Pschierer, *J. Amer. med. Ass.*, 1947, **135,** 1123.
90. Craver, *Radiology* 1948, **50,** 486.
91. Kurnick, Paley, Fieber and Adler, *Ann. intern. Med.*, 1949, **30,** 974.
92. Ben-Asher, *Amer. J. med. Sci.*, 1947, **217,** 162.
93. Kennedy and Aub, *Med. Clin. N. Amer.*, 1949, **33,** 1301.
94. Scheinmel, Roswit and Lawrence, *Radiology,* 1950, **54,** 165.
95. Bauer and Erf, *Amer. J. med. Sci.*, 1950, **219,** 16.
96. Epstein, *Urol. cutan. Rev.*, 1949, **53,** 628.
97. Goldberg and Mason, *Arch. Derm. Syph.*, 1949, **60,** 181.
98. Henstell, Tober and Newman, *Blood,* 1947, **2,** 564.
99. Wilkinson and Fletcher, *Lancet,* 1947, **253,** 540.
100. Iverson and Kjerulf-jensen, *Acta clin. Belge,* 1948, **3,** 138.

101. Spurr, Smith, Block and Jacobson, *J. Lab. clin. Med.*, 1950, **35,** 252.
102. Skinner, Carr and Denman, *J. thorac. Surg.*, 1948, **17,** 428.
103. Alpert, Greenspan and Peterson, *Ann. intern. Med.*, 1950, **32,** 393.
104. Herve, *Acta clin. Belg,* 1948, **3,** 419.
105. Snider, *South. med. J.*, 1948, **41,** 11.
106. Aleksandrowicz, *Le Sang,* 1948, **19,** 94
107. Aleksandrowicz, *Amer. J. med. Sci.*, 1948, **216,** 275.
108. Aleksandrowicz, Blikarski, Legczinski, Slopek and Kowalczkowa. *Polsk Tyg. Lek.*, 1949, **4,** 481.
109. Chasis, Goldring and Baldwin, *J. clin. Invest.*, 1950, **29,** 804.
110. Sousek and Beck, *Čas. Lék. čes.*, 1949, **88,** 1372.
111. Aleksandrowicz, *Polsk. Tyg. Lek.*, 1948, **3,** 1489.
112. Aleksandrowicz, Blikarski, Kostkowski and Hanicki, *Przeg. Lek.*. 1949, **5,** 32.
113. Tsevrenis, Thuilliez and Foussier, *Pr. Méd.*, 1950, **58,** 18.
114. Videboeck, *Ugeskr. Laeg.*, 1949, **111,** 147.
115. Zanes, Doan and Hoster, *J. Lab. clin. Med.*, 1948, **33,** 1002.
116. Brown and Davis, *Glasg. med. J.*, 1950, **31,** 93.
117. Domokos and Kalemen, *Int. Z. Vitaminforsch.*, 1950, **21,** 443.
118. Justin-Besançon, Lamotte-Barrillon and Polonovski, *Sem. Hôp., Paris,* 1948, **24,** 1529.
119. Rosenthal, *Lancet,* 1948, **254,** 408.
120. Conley, Hartman and Morse, *Johns Hopk. Hosp. Bull.*, 1949, **84,** 255.
121. Roswit and Kaplan, *Amer. J. Roentgenol.*, 1949, **61,** 626.
122. Danowski, Greenman, Gow, Weigand, Mateer, Peters, Cosgrove, Seiferth and Davis, *J. Pharmacol.*, 1950, **98,** 147.
123. Bierman, Strait and Hrenoff, *J. nat. Cancer Inst.*, 1949, **10,** 93
124. Frieden and White, *Yale J. biol. Med.*, 1950, **22,** 395.

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THE BIOLOGICAL AND CHEMICAL ASSAY OF TINCTURES OF DIGITALIS

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INTRODUCTION

THE biological assay of digitalis preparations has been attempted by a variety of methods, employing many of the more commonly used experimental animals. Satisfactory agreement has not been obtained between the results of these various biological assays, and this is probably due to the effects of a large number of factors such as the variation in concentration of the different digitalis glycosides in different digitalis preparations, the effects of saponins, the adsorption of the glycosides by heart and skeletal muscle and by serum albumins, and the great variations in absorption, accumulation and excretion of the glycosides which may result from the adsorption effect. The possible effects of these various factors, make it very unlikely that any single method of biological assay, can render a complete picture of the therapeutic value of every preparation of digitalis. Chemical assays in which the digitoxin content, or the mixed glycoside content of digitalis preparations was weighed have been described by Keller[1], Martindale[2] and Tschirch and Wolter[3].

A colorimetric estimation based upon the orange colour developed when suitable solutions of the active principles of digitalis preparations are mixed with an alkaline picrate solution was reported by Baljet[4] in 1918. This reagent, it is claimed, is specific for molecules containing the unsaturated lactone grouping $-C\begin{matrix} \diagup CH_2-C=O \\ \quad\;\; | \\ CH-O \end{matrix}$ found in the aglycones of the digitalis glycosides. The Baljet reaction has been used by Knudson and Dresbach[5], and Bell and Krantz[6] as the basis of a colorimetric assay of digitalis preparations. Hagemeier[7] found that the greatest colour activity, using the Baljet reagent, was given by an inert glycoside diginin, whilst Knudson and Dresbach[5], Ockeloen and Timmers[8] and Carratala[9] found that an assay based on this reaction yielded results which agreed with one or other of the biological assays. Dyer[10], Wasicky and co-workers[11], and Vos and Welsh[12], however, found no agreement between such a colorimetric assay and one or other of the biological assays. Anderson and Chen[13] used a modified Raymond method, based on the blue colour which develops when alcoholic glycosidal solutions are mixed with an alkaline solution of *meta*dinitro-

benzene in alcohol, for the colorimetric assay of digitalis tinctures. Cänback[14] has criticised this assay method. Wasicky[15] has described colour reactions of the digitalis glycosides and aglycones with 3:5-dinitrobenzoic acid, and with sodium nitroprusside by which he could estimate the concentrations of glycoside and of aglycone in solutions where both are present. The results of a colorimetric assay of digitalis preparations would be expected to agree with those of a biological assay, only if the intensity of the colour produced by each glycoside were proportional to the degree of physiological change it produced. Colorimetric and biological assays might conceivably yield comparable results when one is dealing with pure glycosides, but the results of a colorimetric assay of digitalis preparations are not likely to agree consistently with those of a biological assay.

Experimental

I. Investigation of the Baljet reaction

For this investigation, and for the comparative assays carried out as described in the following pages, a standard tincture of digitalis was prepared as follows:—

The Preparation of Tincture A. 10C g. of a coarsely powdered sample of digitalis leaf, containing 11·4 units of activity per g. were continuously extracted for 6 hours in a Soxhlet extractor, with 500 ml. of absolute alcohol. The resulting extract was cooled, adjusted to 500 ml. by the addition of absolute alcohol, allowed to stand for 48 hours and clarified by filtration through coarse filter paper. This tincture represents a 1 in 5 extract of the digitalis leaf.

The Method of Developing the Colour from Various Samples of Tincture A. The volume of tincture used was added to 15 ml. of distilled water and 2 ml. of freshly prepared 12·5 per cent. solution of lead acetate added. The volume was adjusted to 25 ml. with distilled water and filtered through paper. 12·5 ml. of the filtrate was placed in a 25-ml. volumetric flask, and 2 ml. of a 4·7 per cent. solution of disodium phosphate containing 7 molecules of water of crystallisation, was added. The contents of the flask were mixed and the whole diluted with distilled water to 25 ml., mixed, and filtered through paper. This decolorisation process has been described by Knudson and Dresbach[5]. The filtrate was again clarified by filtration through a sintered glass filter number D4, to remove traces of the phosphate precipitate which are not retained by the coarser Whatman No. 1 filter papers, used in the above operations. 12·5 ml. of this final filtrate was placed in a dry-stoppered bottle. Fresh samples of the Baljet reagent were prepared by mixing stock aqueous solutions of sodium hydroxide in 10 per cent. w/v concentration, and of picric acid in 1 per cent. w/v concentration, in the proportions of 5 volumes and 95 volumes respectively. The mixture was then filtered through a sintered glass filter, number D4. 12·5 ml. of the Baljet reagent was added to the 12.5 ml. of glycosidal filtrate in the bottle and mixed. 12·5 ml. of the Baljet reagent was mixed with 12·5 ml. of distilled water

for use as the "control" solution. The intensities of the coloured solutions obtained by this colorimetric method, were compared by means of the "Spekker" photoelectric absorptiometer H 454. The colour filters OB1, supplied with the instrument were used.

The Variation with Time of the Intensity of the Colour Developed in the Baljet Reagent. Bell and Krantz in their original work, measured the intensity of the coloured solutions 20 minutes after mixing the Baljet reagent with the glycoside filtrates. In their later papers they have extended this period from 20 to 40 minutes. The variation of colour intensity with time was therefore investigated. 10 ml. of each of the decolorised filtrates from (a) 1·0 ml., (b) 1·5 ml., (c) 2·0 ml. and (d) 2·5 ml. of tincture A was mixed with 10 ml. of the Baljet reagent. The filtrates and the Baljet reagent were all clarified by filtration through Whatman No. 50 filter papers, 10 ml. of the Baljet reagent was mixed with 10 ml. of distilled water to form the "control." The intensity of the colour developed was measured by comparison with this "control" at intervals of 5 minutes for 90 minutes, and then at intervals of 20 minutes for a

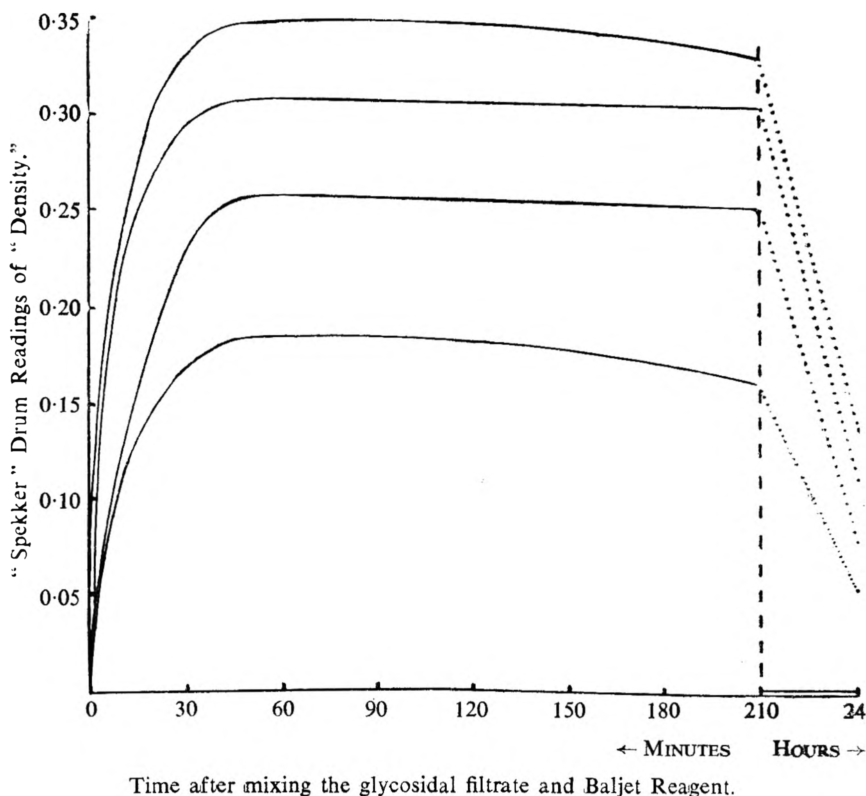


FIG. 1. Effect of time on the colour developed in the Baljet Reaction. Topmost graph, 2·5 ml. of tincture A; middle graphs, 2·0 ml. and 1·5 ml., and the lowest graph, 1·0 ml. of tincture A.

further 2 hours. A final reading was made after 24 hours. The results are shown in Figure 1.

The results presented in Figure 1 show that the difference in intensity of colour between the solutions tested and the reagent control takes 40 to 60 minutes to reach a maximum, after which the readings are fairly stable for about one hour, and fall very slightly over a period of about 3 hours. The more dilute glycosidal solutions differ slightly, as can be seen in the case of the readings for 1·0 ml. of tincture A where the readings fall from about 0·185 to 0·170 quite markedly, within about 3 hours.

After 24 hours, the readings have fallen very considerably. The question arises, whether this fall in the readings is due to a decrease in intensity of the colour of the glycosidal solutions with age, or to an increase in intensity of that of the Baljet reagent with age, or to both effects. In order to study the variation with time, of the intensity of the colour (a) developed from a glycosidal solution and (b) of the Baljet reagent itself, four glycosidal solutions were prepared from four different volumes of tincture A, clarified through a Whatman No. 50 filter paper, and 10 ml. of each mixed with 10 ml. of Baljet reagent. 10 ml. of the Baljet reagent was also mixed with 10 ml. of distilled water to form the Baljet reagent control solution. After 1 hour, readings were made as follows. The two blue filters O.B.1 were used. The orange filter O.Y.2 for the right hand side of the absorptiometer was placed in position. The water cell was placed on the left hand side of the instrument and the absorptiometer set at zero. The orange filter was removed and one of the five coloured solutions, in a 1 cm. path absorptiometer cup, was placed in position. The galvanometer was brought back to zero by closing the right hand light shutter, by turning the calibrated drum. The reading on the drum was taken. The readings were repeated 24 hours later. The results are shown in Table I.

TABLE I

EFFECT OF TIME ON THE COLOUR DEVELOPED IN THE BALJET REACTION

Volume of tincture A in ml.	Difference in " density " between the orange filter O.Y.2 and the solutions under test		Differences in " density " between the intensity of each coloured glycoside solution and the " control "	
	after 1 hour	after 24 hours	after 1 hour	after 24 hours
1·0	0·247	0·337	0·182	0·065
1·5	0·174	0·315	0·255	0·087
2·0	0·130	0·286	0·299	0·116
2·5	0·038	0·249	0·341	0·153
Nil :— " Control " ...	0·429	0·402		

The results given in Table I and Figure 1 show that the intensity of the colour developed from these glycosidal solutions when mixed with alkaline picrate solution, rises to a maximum in 40 to 60 minutes and

begins to decrease 2 or more hours after mixing. The intensity decreases very markedly during the 24 hours after mixing. The intensity of the colour of the alkaline picrate reagent is reasonably constant during the 24 hour-period following its preparation, the intensity of the colour increasing very slightly. It was decided to make all Spekker readings of "density" 1 hour after mixing the glycosidal filtrates with the Baljet reagent.

The Preparation of Calibration Curves for Tincture A. Method A. The results obtained using the method of colour development described on page 881 are shown in graphical form in Figure 2. In most cases each point plotted represents the average of 10 individual estimations of "density" for the volume of tincture A used.

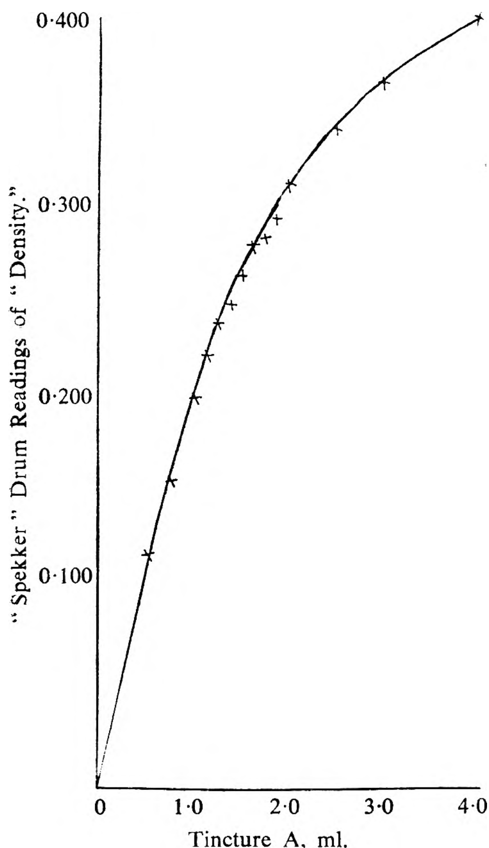


FIG. 2. Calibration curve for tincture A using method A.

Method B. Method A corresponds in essentials with the method described by Bell and Krantz. It can be seen that the increase in the intensity of the colour developed becomes less as the volume of tincture used increases, so that volumes of about 3·0 ml. or more of a normal pharmacopœial tincture cannot be estimated colorimetrically with a high degree of accuracy. For this reason a second calibration curve was prepared in which the final coloured solutions were diluted with an equal volume of distilled water before estimating the intensity of colour by means of the Spekker absorptiometer. The procedure was as follows:—

1. The final filtrates from the decolorisation process were filtered through acid-washed Whatman No. 50 filter papers.

2. 10 ml. of each filtrate was mixed with 10 ml. of the Baljet reagent, also filtered through a Whatman No. 50 filter paper.

3. 20 ml. of the "control" solution was prepared by mixing 10 ml. of the Baljet reagent and 10 ml. of distilled water.

4. The colours were allowed to develop for 1 hour.

5. Each of the coloured solutions including the "control" solution, was then diluted with 20 ml. of distilled water.

6. Immediately after dilution, the intensity of the colour of each of the glycosidal solutions was estimated by comparison with the diluted "control" solution.

The results are presented in graphical form in Figure 3. Each point represents the average of 20 individual estimations of "density" for the volume of tincture A used. The standard deviation of the results using these 2 methods was about the same, and varied between 2 per cent. and 8 per cent., so that there is free choice of method. The method B has been used by the authors, since, as can be seen from Figures 2 and 3, the curvature of the calibration curve is much less in the case of Figure 3 for the larger volumes of tincture A.

II. The comparison of the potencies of tinctures X and A using the colorimetric method

The Preparation of Tincture X. This tincture was prepared by macerating for 2 days with 500 ml. of alcohol (70 per cent.), 50 g. of the same sample of powdered digitalis leaf as was used to prepare tincture A. The liquid was then strained off, the marc pressed, and the expressed liquid mixed with the liquid strained from the marc—allowed to stand for 48 hours and clarified by filtration through coarse filter paper.

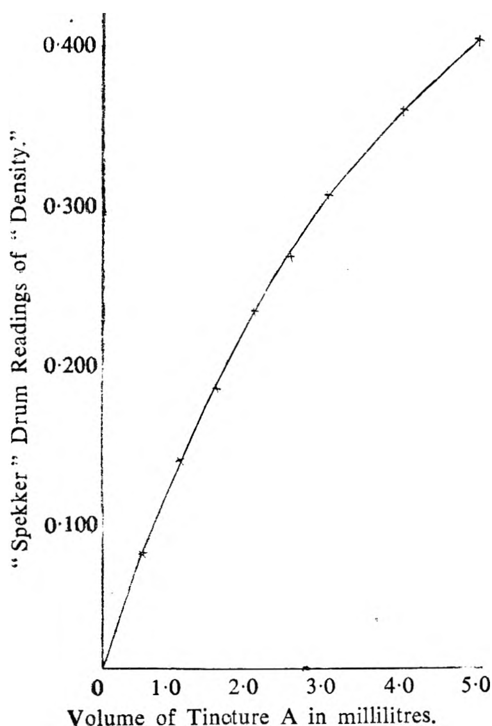


FIG. 3. Calibration curve for tincture A using method B.

The Comparison of the Colour Intensities developed by Tinctures X and A. Five comparisons were made using for each:—(1) Two volumes of tincture X, (2) Two volumes of tincture A, (3) One volume of tincture A. Each volume of tincture was decolorised by the method of Knudson and Dresbach (page 881) and the intensities of the coloured solutions which were produced when the decolorised filtrates were mixed with the Baljet reagent, were compared by the method B described above.

The Calculation of Relative Potency. The potency of tincture X

expressed as a percentage of tincture A was calculated from the formula:-

$$50\left(\frac{R_X - R_{100}}{R_{100} - R_{50}}\right) + 100,$$

where R_X = the reading of "density" for the coloured solution delivered from two volumes of tincture X, R_{100} = the reading of "density" for the coloured solution developed from two volumes of tincture A, and R_{50} = reading of "density" for the coloured solution developed from one volume of tincture A. This formula was used by Bell and Krantz[1] in their colorimetric assay. The volumes of tinctures used by them were:—5·0 ml. of tincture of unknown strength, 5·0 ml. of the U.S.P. Reference Standard Powder Tincture, and 5·0 ml. of the U.S.P. Reference Standard Powder Tincture, diluted with an equal volume of alcohol (71 per cent.). The results of the five assays are shown in Table II.

TABLE II

COMPARISON BY THE COLORIMETRIC METHOD OF TINCTURES A AND X

Assay number	Volume of tincture X used, in ml.	Reading of "Density" (R_X)	Volume of tincture A used, in ml.	Reading of "density" (R_{100})	Volume of tincture A used, in ml.	Reading of "density" (R_{50})	The percentage potency of tincture X relative to the potency of tincture A as obtained from the Bell & Krantz formula
1	1·0	0·078	1·0	0·141	0·5	0·077	50
2	2·0	0·135	2·0	0·233	1·0	0·136	50
3	3·0	0·183	3·0	0·309	1·5	0·185	49
4	4·0	0·230	4·0	0·360	2·0	0·233	49
5	5·0	0·263	5·0	0·405	2·5	0·262	50

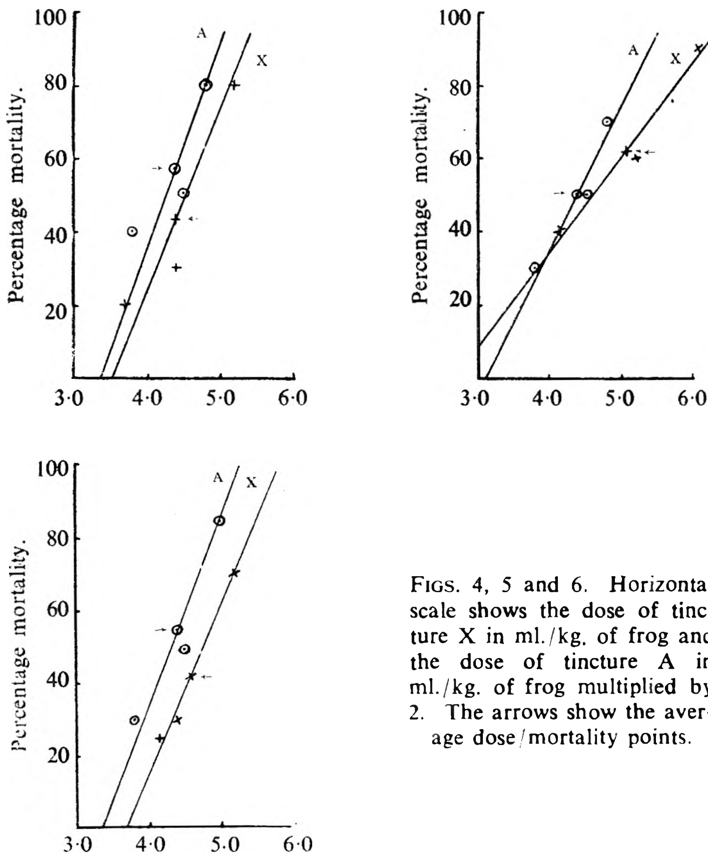
$$\text{The mean ratio } \frac{\text{potency of tincture X}}{\text{potency of tincture A}} = \frac{0\cdot5}{1\cdot0}$$

III. THE COMPARISON OF THE POTENCIES OF TINCTURES A AND X BY BIOLOGICAL METHODS USING FROGS

Three assays using the method described by Møller[16]. Six groups of frogs were chosen for each assay, and 3 dilutions in 0·6 per cent. saline solution of each of the 2 tinctures were made so that the expected mortalities for the 3 doses of each tincture were about 20 per cent., 50 per cent., and 70 per cent. Each of the dilutions was made so that the dose volume was 0·02 ml. per g. of frog. Injection was made into the ventral lymph sac, the needle being inserted in the thigh and directed upwards to the lymph sac so as to pierce a small portion of the thigh muscle. The frogs were inspected after 18 hours, and the mortality ratio for each dose value was recorded.

The average dose value, and the average mortality ratio for each tinc-

ture were calculated from the results obtained, and these figures, along with the 3 appropriate experimental results were used to plot a dose/mortality line for each tincture, which was drawn by eye to pass through the average dose/mortality point. The doses of the tinctures used, in ml. per kg. of frog, form the abscissæ and the percentage mortalities of the frogs form the ordinates. From these two lines, the dose of each tincture corresponding to 50 per cent. mortality, i.e., the LD50, was read off, and the ratio between the LD50's of the 2 tinctures was recorded as the ratio of their potencies. The results of these three assays are recorded in Tables III, IV and V and Figures 4, 5 and 6. In these figures, the volumes of tincture A used have been multiplied by 2 since tincture A is a 1 in 5 extract, and tincture X is a 1 in 10 extract of the sample of leaf.



FIGS. 4, 5 and 6. Horizontal scale shows the dose of tincture X in ml./kg. of frog and the dose of tincture A in ml./kg. of frog multiplied by 2. The arrows show the average dose/mortality points.

The Calculation of Regression Equations for Tinctures A and X. Owing to the poor and irregular supply of frogs during the past years, it was decided that any suitable frogs left over from the comparative assays, and too few for a complete assay, should be used to obtain additional dose/mortality readings. The combined results could then be used

TABLE III

BIOLOGICAL ASSAY No. 1, CARRIED OUT ON SIX GROUPS OF TEN FROGS

Tincture used	Doses of tinctures in ml./kg. of frog	Mean dose in ml./kg. of frog.	Number of deaths	Mortality, per cent.	Mean mortality, per cent.
X	3·7		2/10	20	
X	4·4	4·4	3/10	30	43
X	5·2		8/10	80	
A	1·9		4/10	40	
A	2·25	2·2	5/10	50	57
A	2·4		8/10	80	

TABLE IV

BIOLOGICAL ASSAY No. 2, CARRIED OUT ON SIX GROUPS OF TEN FROGS

Tincture used	Doses of tinctures in ml./kg. of frog	Mean dose in ml./kg. of frog	Number of deaths	Mortality, per cent.	Mean mortality, per cent.
X	4·15		4/10	40	
X	5·2	5·1	6/10	60	58
X	6·06		9/10	90	
A	1·9		3/10	30	
A	2·25	2·2	5/10	50	50
A	2·4		7/10	70	

TABLE V

BIOLOGICAL ASSAY No. 3, CARRIED OUT ON SIX GROUPS OF TWENTY FROGS

Tincture used	Doses of tinctures in ml./kg. of frog	Mean dose in ml./kg. of frog	Number of deaths	Mortality per cent.	Mean mortality, per cent.
X	4·15		5/20	25	
X	4·4	4·6	6/20	30	42
X	5·2		14/20	70	
A	1·9		6/20	30	
A	2·25	2·2	10/20	50	55
A	2·5		17/20	85	

TABLE VI

COMBINED RESULTS OF THE THREE BIOLOGICAL ASSAYS

Assay number	LD50 of Tincture X in ml./kg. of frog	LD50 of Tincture A in ml./kg. of frog	Potency of Tincture X / Potency of Tincture A
1	4·55	2·15	0·47
2	4·6	2·2	0·48
3	4·8	2·15	0·45

$$\text{The average ratio } \frac{\text{potency of tincture X}}{\text{potency of tincture A}} = 0{\cdot}47$$

to calculate a regression line. The method described by Finney[17] was used.

(i) *The calculation of a regression equation for Tincture X.* The combined results obtained for tincture X are shown below (Table VII).

TABLE VII

BIOLOGICAL ASSAY OF TINCTURE X

Dose of tincture X in ml./kg. of frog	(X) Log of dose	(n) No. of animals injected	(r) No. of animals which died	Mortality, per cent.	Probit Y
3·7	0·5682	27	4	14·7	3·95
4·15	0·6180	30	9	30	4·47
4·4	0·6435	39	14	36	4·64
5·2	0·7160	47	31	66	5·41
6·06	0·7825	10	9	90	6·28

The final regression equation was:—$y = 4{\cdot}84 + 10{\cdot}22\ (x - 0{\cdot}685)$

$= 10{\cdot}22x - 1{\cdot}84.$

The log. LD50 = m = 0·67.

$\chi^2_{(3)} = 0{\cdot}19$ $\quad V_{(m)} = 0{\cdot}000117$

$V_{(b)} = 3{\cdot}52$ $\quad \sigma_{(m)} = 0{\cdot}0108$

and $\sigma_{(b)} = 1{\cdot}88$

The fiducial limits for the LD50 of tincture X at the 95 per cent. probability level are 4·45 ml. and 4·91 ml./kg. of frog. The best estimate is 4·68 ml./kg. of frog.

(ii) *The calculation of a regression equation for tincture A.* The combined dose/percentage mortality results for tincture A are shown in Table VIII.

TABLE VIII

RELATION BETWEEN DOSAGE AND THE PERCENTAGE MORTALITY IN THE BIOLOGICAL ASSAY OF TINCTURE A

Dose of tincture A in ml./kg. of frog	(x) Log dose	(n)	(r)	Mortality, per cent.	Probit Y
1·90	0·2788	48	17	35·4	4·39
2·25	0·3522	60	34	56·6	5·12
2·41	0·3820	40	29	73	5·42
2·50	0·3979	34	27	79·4	5·58

The equation for the regression line is:—$y = 5{\cdot}25 + 9{\cdot}46\ (x - 0{\cdot}35)$

$= 9{\cdot}46x + 1{\cdot}95$

$\sigma_{(b)} = 2{\cdot}22$ $\quad \sigma_{(m)} = 0{\cdot}0119$

$V_{(b)} = 4{\cdot}92$ $\quad V_{(m)} = 0{\cdot}00014$

$\chi^2_{(2)} = 0{\cdot}676$ $\quad m = 0{\cdot}3229$

The fiducial limits for the LD50 of tincture A at the 95 per cent. probability level are:—

1·95 ml. and 2·20 ml./kg. of frog.

The best estimate for the LD50 is 2·10 ml. of tincture A per kg. of frog.

(iii) *The calculation of the relative potency of Tinctures A and X from their regression equations.* The common slope $b_{(AX)}$ of the regression lines for the two tinctures is $b_{(AX)} = 9 \cdot 9 \pm 1 \cdot 4$.

A χ^2 for parallelism was calculated to be 0·073. There is one degree of freedom, and this figure is not significant. There is no evidence of any conflict with the hypothesis that the two lines are parallel.

After inserting the figure $b_{(AX)}$ into their regression equations, new estimates for the log. LD50 (m) of tinctures A and X, were obtained:—

$$m_{(A)} = 0 \cdot 324$$
$$m_{(X)} = 0 \cdot 674.$$

From these values, the fiducial limits for the potency of tincture X with respect to tincture A—the standard tincture—were calculated to be 0·41 and 0·48, at the 95 per cent. probability level. The best estimate for the ratio $\frac{\text{potency of tincture X}}{\text{potency of tincture A}}$ is 0·45.

IV. The comparison of the results of the biological and chemical assays of tinctures A and X

The results for the ratio $\frac{\text{potency of tincture X}}{\text{potency of tincture A}}$ are (i) by the chemical assay, 0·50; (ii) by the bio-assay of Møller, 0·47; (iii) by the comparison of regression equations for the frog mortality experiments, 0·45. If the third result is taken to be the most accurate, since it is based on the reactions of 335 frogs, then the figure obtained by the chemical assay differs by about 11 per cent., and the figure obtained by Møller's assay differs by about 4 per cent. On the whole, there is good agreement between the results of the three assay methods. The use of the simple dose/mortality line, as employed by Møller, is open to some criticism. Gaddum[18] and many other workers have described the advantages of the use of the probit, and of the logarithm of the dose volumes. It was therefore decided to use the Møller assay only for a preliminary estimation of relative potency, and to employ the type of assay described by Gaddum[18] where the slope of the probit/log dose curve is determined in each experiment.

(a) *The Effect of Saponins on the Biological and Colorimetric Assays of Digitalis Preparations.* The discrepancy between the results of cat and frog assays, in the comparison of two tinctures of digitalis may be due to the presence of digitalis saponins in one of the tinctures, since saponins are reported to be much more toxic to cold-blooded animals than they are to warm-blooded animals. Saponins may also be responsible for the discrepancy between the results of the frog and the colorimetric assays, reported by many workers, since the digitalis saponins do not give a positive reaction with the Baljet reagent, as they do not contain an unsaturated lactone grouping in the sapogenin part of their molecules.

(i) *Test for the Presence of Saponin in the Sample of powdered Digitalis leaf, used in the Preparation of Tinctures A and X.* A 1 in 10 infusion

was prepared from 5 g. of the powdered digitalis leaf. To the filtered infusion, sufficient sodium chloride was added to produce a concentration of 0·9 per cent. To 2·0 ml. of this solution in a test tube 2·0 ml. of a 1 in 40 dilution in normal saline of washed horse red blood cells was added. The contents of the test tube were mixed, and re-mixed after 15 minutes—allowed to stand for 24 hours and inspected for hæmolysis. This test was repeated with 1·0 ml., 0·5 ml. and 0·25 ml. volumes of the saline-infusion solution. In all cases the volume was adjusted to 2·0 ml. with normal saline, before adding the blood dilution. Hæmolysis occurred in all cases.

(ii) *The Preparation of Tinctures I, J, O and P.* From the same sample of powdered digitalis leaf as was used to prepare tinctures A and X the following tinctures were prepared.

Tincture I. This was a 1 in 10 tincture prepared by the continuous extraction of 100 g. of the powdered leaf, with 1000 ml. of absolute alcohol in a Soxhlet extractor for six hours. The resulting extract was adjusted to 1000 ml. by the addition of absolute alcohol, and clarified after standing for 48 hours, by filtration through coarse filter paper.

Tincture J. 50 g. of the powdered leaf was macerated for 2 days with 500 ml. of alcohol (70 per cent.) according to the pharmacopœial method. The resulting tincture was clarified by filtration through coarse filter paper, after standing for 48 hours. Pénau and Hardy[19] and Mellanoff[20] have reported that digitonin forms an insoluble complex with various sterols, of which the cholesterol-digitonin complex is the least soluble. Pénau and Hardy describe the preparation of a digitonin-ergosterol complex, which can be used for the quantitative estimation of ergosterol. The solution in which the complex forms has an alcoholic strength of about 90 per cent. This process was used as a basis for the removal of saponin from a sample of tincture J as follows.

Tincture O. 100 ml. of tincture J was mixed, in a 500-ml. stoppered bottle, with 100 ml. of absolute alcohol, and 100 ml. of a 0·175 per cent. solution of ergosterol in absolute alcohol was added. This yields a mixture of approximately 90 per cent. alcoholic strength. The mixture was allowed to stand for 18 hours, at room temperature, and the bulky precipitate of saponin-ergosterol complex, and other precipitated matter, was filtered off through a sintered glass filter, and the bottle and filter washed 3 times with a total of 50 ml. of absolute alcohol. (The precipitate was later washed with 3 20-ml. quantities of alcohol (70 per cent.). These washings when evaporated yielded only a slight Keller-Kiliani reaction.) The filtered solution and washings were then reduced to about 70 ml. by distillation under reduced pressure at a temperature not exceeding 60°C. The contents of the distillation flask were removed and adjusted to 100 ml. with the washings of the distillation flask. Absolute alcohol was used for these washings. The final 100 ml. of treated tincture was filtered through coarse paper after being allowed to stand for 7 days.

Tincture P. 100 ml. of tincture J was mixed in a 500-ml. stoppered

bottle with 200 ml. of absolute alcohol and allowed to stand for 18 hours. The mixture was then filtered through a sintered glass filter, the bottle and filter washed with 50 ml. of absolute alcohol and the filtrate and washings evaporated, as in the case of tincture O, to yield a final volume of 100 ml. This was allowed to stand for 7 days and filtered through a coarse filter paper. Tincture P was prepared as a control for tincture O.

(iii) *Test for the Presence of Saponin in Tinctures of Digitalis.* Tinctures A, X, I, J, O and P were tested for saponin by the following method. 20 ml. of each tincture was evaporated to dryness in an evaporating dish, heated on a water-bath. The residue was thoroughly mixed with 40 ml. of normal saline solution and the mixture filtered. It was found that those tinctures which were later shown to contain saponin yielded a residue which very easily passed into solution when saline solution was added. Such solutions could be filtered clear only with difficulty. The saponin-free tinctures yielded a residue which mixed with saline only with difficulty and could be clarified very easily—filtration through Whatman No. 1 filter papers was efficient. In each case, test tubes were set up containing (1) 5·0 ml., (2) 1·0 ml., (3) 0·5 ml., (4) 0·25 ml., (5) 0·125 ml., (6) 0·05 ml., (7) 0·025 ml. and (8) 0·01 ml. of filtrate, the latter quantities being obtained by dilution of the primary filtrate with saline. The volume of the liquid in tubes 3 to 8 inclusive was adjusted to 1·0 ml. by the addition of normal saline. To each tube was added 1·0 ml. of a 1 in 40 dilution in normal saline of washed horse red blood cells. Each tube was mixed and re-mixed 15 minutes later, allowed to stand for 24 hours and inspected for hæmolysis by viewing from above against a white background. Where hæmolysis had occurred, the solution was clear and red—the deposit if any was fine, and white, or grey, not red—this is due to red cell stroma. Where no hæmolysis occurred the deposit was red, and on shaking the mixture became opaque. The results are shown in Table IX.

TABLE IX

RESULTS OF HÆMOLYSIS TEST FOR THE PRESENCE OF SAPONIN IN TINCTURES A, X, I, J, O AND P

Millilitres of filtrate	A	X	I	J	O	P
5·0	—	*	—	*	—	*
1·0	—	*	—	*	—	*
0·5	—	*	—	*	—	*
0·25	—	*	—	*	—	*
0·125... ...	—	*	—	*	—	*
0·05	—	*	—	*	—	*
0·025... ...	—	slight	—	*	—	slight
0·01 ...	—	?	—	?	—	—

* indicates hæmolysis.

Thus 0·025 ml. of filtrate from tinctures X, J and P just cause hæmolysis, and since the filtrates are 1 in 2 dilutions of the original tinctures, this means that 0·0125 ml. of the original tinctures will just cause hæmolysis under these conditions. The total final volume is 2·0 ml., the Hæmolytic Index of these three tinctures is therefore about $\frac{1 \times 2}{0 \cdot 0125} = 160$. In the case of tinctures A, I and O no hæmolysis occurred even when 5·0 ml. of filtrate were used, and the hæmolytic index must therefore be less than 2·4. This shows that there is a clearly defined difference in saponin content between tinctures A, O and I on the one hand, and tinctures X, J and P on the other. With regard to tincture O, and its control tincture P, these results show that the method employed for the removal of saponin is efficient. With regard to tinctures A, I and J, these results indicate that absolute alcohol will extract a negligible amount of saponin, whereas alcohol (70 per cent.) will extract appreciable amounts from a leaf which contains saponin.

(b) *The Biological and Colorimetric Assays of Tinctures I, J, O and P.*

(i) *The biological comparison of tinctures I and J.* Six groups each of 10 frogs were injected with appropriate doses of the 3 tinctures following the method described by Møller. The ratio $\frac{\text{potency of Tincture J}}{\text{potency of tincture I}}$ was found to be 0·84.

Three further comparisons were made using the method described by Gaddum[18].

Assay No. 1. Four groups, each of 10 frogs, were used. The ratio $\frac{\text{potency of tincture J}}{\text{potency of tincture I}}$ was found to be 0·86. The limits within which this ratio lies, at the 95 per cent. probability level, corresponding to $\pm$ 1·96 standard deviations, were calculated to be 0·79 and 0·95. The weight of the estimate was calculated to be 2158.

Assay No. 2. Four groups each of 10 frogs were used. The ratio $\frac{\text{potency of tincture J}}{\text{potency of tincture I}}$ was found to be 0·81. The limits within which this ratio lies at the 95 per cent. probability level were calculated to be 0·75 and 1·09. The weight of the estimate was 853.

Assay No. 3. Four groups each of 25 frogs were used. The ratio $\frac{\text{potency of tincture J}}{\text{potency of tincture I}}$ was found to be 0·89. The limits within which this ratio lies at the 95 per cent. probability level were calculated to be 0·83 and 0·96. The weight of the estimate was 3,415.

The weighted mean of the 3 results obtained in these assays, for the ratio $\frac{\text{potency of tincture J}}{\text{potency of tincture I}}$ was calculated to be 0·87. The total weight of this estimate was 6426 and, at the 95 per cent. probability level, the

limits within which the weighted mean of the results of the three assays lay, were 0·85 and 0·90.

C. As before (page 887), the combined results from the above assays were used to calculate regression equations for tinctures I and J as shown below.

Tincture I. Altogether 186 frogs were used to prepare a regression line for tincture I. The regression equation was calculated to be $y = 11 \cdot 04x - 1 \cdot 51$. The best estimate of the LD50 was found to be 3·9 ml of tincture I per kg. of frog. The fiducial limits of the LD50 at the 95 per cent. probability level were 3·7 ml. and 4·1 ml. per kg. of frog.

Tincture J. Altogether 133 frogs were used to prepare the regression line. The regression equation was calculated to be $y = 11 \cdot 34x - 2 \cdot 51$. The best estimate of the LD50 was 4·6 ml. of tincture J per kg. of frog. The fiducial limits of the LD50 at the 95 per cent. probability level were 4·4 ml. and 4·9 per ml./kg. of frog. A calculation of the relative potency of these 2 tinctures from the above data is made below: page 895.

(ii) *The biological comparison of tinctures O and P.* Three comparisons were made using the method described by Gaddum[18].

Assay No. 1. Four groups, each of 10 frogs, were used. The ratio $\frac{\text{potency of tincture O}}{\text{potency of tincture P}}$ was found to be 1·03. The limits within which this ratio lies at the 95 per cent. probability level, were calculated to be 0·88 and 1·21. The weight of the estimate was 787.

Assay No. 2. Four groups, each of 10 frogs, were used. The ratio $\frac{\text{potency of tincture O}}{\text{potency of tincture P}}$ was found to be 0·99. The limits within which this ratio lies at the 95 per cent. probability level were calculated to be 0·85 and 1·16. The weight of the estimate was 886.

Assay No. 3. Four groups, each of 15 frogs, were used. The ratio $\frac{\text{potency of tincture O}}{\text{potency of tincture P}}$ was found to be 0·96. The limits within which this ratio lies at the 95 per cent. probability level were calculated to be 0·82 and 1·11. The weight of the estimate was 986.

The weighted mean of the three results for the ratio $\frac{\text{potency of tincture O}}{\text{potency of tincture P}}$ was calculated to be 1·00. The limits within which this ratio lies at the 95 per cent. probability level were calculated to be 0·92 and 1·09. The total weight of the estimate was 2659.

The combined results from the above assays were used to calculate a regression equation for tincture O.

Altogether 107 frogs were used to calculate the regression equation. The equation for the regression line was calculated to be $y = 9 \cdot 32x - 1 \cdot 37$. The best estimate of the LD50 of tincture O was calculated to be 4·8

ml./kg. of frog. The fiducial limits for the LD50 at the 95 per cent. probability level were 4·5 ml. and 5·4 ml. of tincture O per kg. of frog.

The combined results from the above assays were used to calculate a regression equation for tincture P. Altogether 123 frogs were used to calculate the regression line. The regression equation was calculated to be $y = 10{\cdot}59x - 1{\cdot}94$. The best estimate of the LD50 of tincture P was 4·5 ml. per kg. of frog. The fiducial limits for the LD50 of tincture P at the 95 per cent. probability level were 4·3 ml. and 4·8 ml./kg. of frog.

(iii) *The comparison of the potencies of tinctures I, J, O and P from their regression equations.* The common slope "b" of the regression lines for the four tinctures is

$$b = 10{\cdot}57 \pm 1{\cdot}39.$$

A χ^2 test for parallelism was calculated to be 0·26. There are three degrees of freedom, so that this figure is not significant, and there is no evidence of any conflict with the hypothesis that the 4 lines are parallel.

New estimates for the log. LD50 (m) were obtained from the four regression equations into which the figure for the common slope (10·57) had been inserted:—$^{m}(I) = 0{\cdot}590$, $^{m}(J) = 0{\cdot}660$, $^{m}(O) = 0{\cdot}680$, $^{m}(P) = 0{\cdot}656$.

From these values the following ratios were calculated:—

(a) The potency of tincture J in terms of tincture I.
(b) The potency of tincture O in terms of tincture I.
(c) The potency of tincture P in terms of tincture I.
(d) The potency of tincture O in terms of tincture P.

The results are shown in table X.

TABLE X

RELATIVE POTENCIES OF TINCTURES I, J, O AND P

Potency of tincture in terms of tincture I or P	Fiducial limits at the 95 per cent. probability level	Best estimate
(a) Tincture J...	79·3 to 90·2 per cent.	84·6 per cent.
(b) Tincture O...	75·5 to 86·7 per cent.	81 per cent.
(c) Tincture P...	77·8 to 94·7 per cent.	85·9 per cent.
(d) Tincture O expressed as a per cent. of tincture P.	87·6 to 101·5 per cent.	94·3 per cent.

The figures for the relationship between tinctures O and P indicate that tincture O is some 5 per cent. weaker than tincture P. This difference in potency falls within the limits of experimental error.

The removal of saponin from this sample of Tincture of Digitalis has caused no significant difference in the potency of the saponin-free tincture as estimated by this method of biological assay. The combined results of the biological assays, for the ratios of the potencies of tinctures I, J, O and P, are shown in Table XI.

TABLE XI

RATIOS OF TINCTURES I, J, O AND P, OBTAINED BY THE DIFFERENT PROCEDURES

Method of calculation	Ratio of potencies of tinctures :— J/I	O/I	P/I	O/P
Møller Assay	0·84	—	—	—
Gaddum Assay	0·87	—	—	1·00
Regression Equations	0·85	0·81	0·86	0·94

(iv) *The colorimetric comparison of tinctures I, J, O and P. The estimation of the relative potency of Tinctures I and J using the Bell and Krantz formula.* Three assays were carried out using for each: (a) 2 ml. of tincture J, (b) 2 ml. of tincture I, (c) 1 ml. of tincture I. Each volume of tincture was decolorised by the method of Knudson and Dresbach. Final clarification was effected by means of Whatman acid-washed No. 50 filter papers. The colours were developed, diluted and their intensities compared by the method B described on page 884. Three more assays were carried out in the same way using: (a) 3 ml. of tincture J, (b) 3 ml. of tincture I, (c) 1·5 ml. of tincture I.

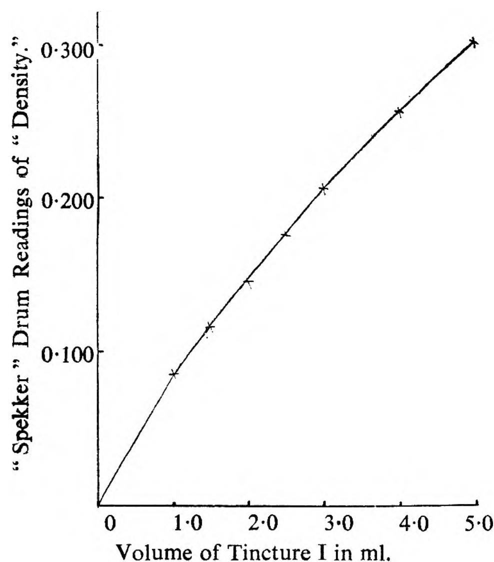


FIG. 7. Calibration curve for Tincture I.

For each of these 6 assays, the intensity of the colour developed from tincture J was compared with that developed from tincture I by means of the Bell and Krantz formula (page 886). The results are shown in Table XII, the potency of tincture being expressed, not as a percentage of that of tincture I, as in the Bell and Krantz formula, but as the corresponding ratio, in which the potency of tincture I is regarded as unity.

2. *The Estimation of the Relative Potency of Tinctures I and J by the comparison of Calibration Curves for the two Tinctures. The calibration curve for tincture I.* A "density" calibration curve was contructed for tincture I. In most cases 10 separate experiments were carried out for each volume of tincture, and the colours were developed, diluted, and estimated as described on page 884. The results are shown in graphical form in Figure 7.

The calibration curve for tincture J. The combined results for tincture J are shown in graphical form in Figure 8. A comparison of the two curves (Figures 7 and 8) at the 0·100, 0·200 and 0·150 " density " levels, indicates that the mean ratio $\frac{\text{potency of Tincture J}}{\text{potency of tincture I}} = 0{\cdot}87$.

TABLE XII

POTENCY OF TINCTURE J EXPRESSED IN TERMS OF TINCTURE I AS IN THE BELL AND KRANTZ FORMULA

Assay No.	Tincture J Volume in ml.	Tincture J Reading of " density "	Tincture I Volume in ml.	Tincture I Reading of " density "	Tincture I Volume in ml.	Tincture I Reading of " density "	Ratio :— Potency of tincture J / Potency of tincture I
1	2·0	0·133	2·0	0·150	1·0	0·088	0·86
2	2·0	0·129	2·0	0·153	1·0	0·083	0·86
3	2·0	0·125	2·0	0·145	1·0	0·084	0·84
4	3·0	0·186	3·0	0·207	1·5	0·119	0·88
5	3·0	0·182	3·0	0·205	1·5	0·115	0·87
6	3·0	0·188	3·0	0·212	1·5	0·120	0·87

Average ratio $\frac{\text{Potency of tincture J}}{\text{Potency of tincture I}} = 0{\cdot}86$

3. *The Comparison of Tinctures O and I Using the Bell and Krantz Formula.* Six assays were carried out by the method used to compare tinctures J and I, and described above. The mean ratio of the six assays was calculated to be $\frac{\text{potency of tincture O}}{\text{potency of tincture I}} = 0{\cdot}86$.

4. *The Comparison of Tinctures P and I Using the Bell and Krantz Formula.* Six assays were carried out as described above. The mean ratio of the 6 assays was calculated to be: $\frac{\text{potency of tincture P}}{\text{potency of tincture I}} = 0{\cdot}88$.

5. *The Results Obtained for the Colorimetric Comparison of the Potencies of Tincture I, J, O and P.* These are shown in Table XIII. As can be seen from the result in column 5, the removal of saponin from a saponin-containing tincture of digitalis has no effect on the chemical

TABLE XIII

RESULTS OF COLORIMETRIC ASSAY OF TINCTURES I, J, O AND P

(1) Method of calculation employed	Ratios of Potencies of tinctures :— (2) J/I	(3) O/I	(4) P/I	(5) O/P from the results shown in columns (3) and (4)
The Bell and Krantz formula ...	0·86	0·86	0·88	$\frac{0{\cdot}86}{0{\cdot}88} = 0{\cdot}98$
Comparison of calibration curves ...	0·87	—	—	—

assay of the tincture when a method employing the Baljet reagent is used.

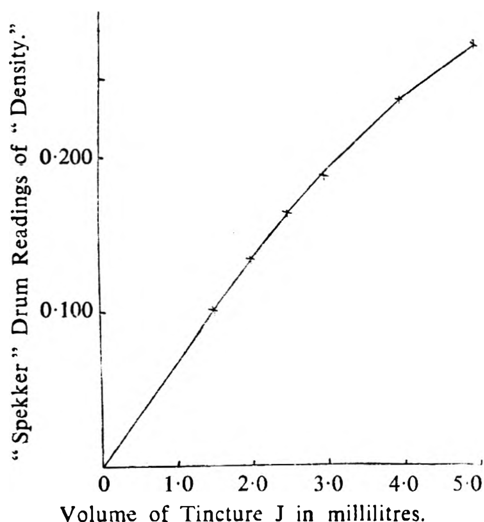


FIG. 8. Calibration curve for Tincture J

(v) *The Comparison of the Results of the Biological and Chemical Methods of Assay for Tinctures I, J. O and P.* A comparison of the results recorded in Tables XI and XIII shows that the chemical and biological comparisons agree closely in the cases of tinctures J and I and of tinctures P and I. In the comparisons of tinctures O and I there is a difference of about 6 per cent. between the result of the colorimetric assay using the Bell and Krantz formula and the result obtained by the comparison of regression equations for the frog mortality experiments. This difference is again apparent in the comparison of the biological and chemical assays of tinctures O and P, when the result of the "regression" method is compared with that of the chemical method. The result of the Gaddum assays, however, agrees closely with that of the chemical method. It can be said in conclusion that the removal of saponin from a saponin-containing tincture of digitalis has no appreciable effect on its potency as estimated by the method of assay using the frog described above, or as estimated by the colorimetric method using the Baljet reagent. The results of the "frog" assays and of the colorimetric assays agree quite well, but it should be remembered that all 6 of the tinctures compared—A, X, I, J, O and P—were prepared from the same sample of powdered Digitalis Leaf.

SUMMARY

1. The comparison of the potencies of tinctures of digitalis, by a chemical method employing the Baljet reaction, has been investigated. Several modifications have been employed, and a method in which the final coloured solutions were diluted with an equal volume of distilled water has been preferred. The period during which the colours are allowed to develop has been extended to 60 minutes, and a method of calculation using the Bell and Krantz formula has been used.

2. Biological assays using the methods described by Møller and by Gaddum have been employed, and regression equations have been calculated. The results of these biological assays agree amongst themselves, and also agree fairly well with the results of the chemical assays.

3. The effect of the removal of saponins from a saponin-containing tincture of digitalis has been investigated. Both biological and chemical methods of comparison indicate that the removal of saponin has no effect on the potency of a saponin-containing tincture of digitalis.

4. All the tinctures used in these comparisons were prepared from the same sample of powdered digitalis leaf. It is not claimed that the results of a chemical assay such as the one employed would agree with the results of a biological assay if the potencies of tinctures prepared from various samples of digitalis leaf were compared.

REFERENCES

1. Keller, *Schweiz. Wchschr. Chem. Pharm.*, 1897, No. 26-28.
2. Martindale, *Pharm. J.*, 1912, **89**, 745.
3. Tschirch and Wolter, *Schweiz. Apoth.-Ztg.*, 1918, **56**, 469.
4. Baljet, *ibid.*, 1918, **56**, 71.
5. Knudson and Dresbach, *J. Pharmacol.*, 1922, **20**, 205.
6. Bell and Krantz, *J. Pharmacol.*, 1945, **83**, 213.
7. Hagemeier, *Arch. exp. Path. Pharmak.*, 1940, **194**, 195.
8. Ockeloen and Timmers, *Pharm. Weekbl.*, 1931, **68**, 820.
9. Carratala, *Semana med.*, 1935, **2**, 154.
10. Dyer, *Quart. J. Pharm. Pharmacol.*, 1932, **5**, 172.
11. Wasicky, Lasch and Schonovski, *Arch. Pharm. Chem.*, 1926, **1**, 92-8.
12. Vos and Welsh, *J. Pharmacol.*, 1945, **85**, 346.
13. Anderson and Chen, *J. Amer. pharm. Ass., Sci. Ed.*, 1946, **35**, 353.
14. Canbäck, *Svensk farm. Tidskr.*, 1947, **51**, 261.
15. Wasicky, *Sci. pharm.*, 1947, **2**, 29.
16. Møller, *Quart. J. Pharm. Pharmacol.*, 1936, **9**, 7.
17. Finney, *Probit Analysis*, Cambridge University Press, 1937.
18. Gaddum, *Spec. Rep. Ser. med. Res. Coun., Lond.*, 1933, No. 183.
19. Penau and Hardy, *J. Pharm. Chim.*, 1929, **9**, 145.
20. Mellanoff, *Amer. J. Pharm.*, 1927, **99**, 390.

DISCUSSION

This paper was read in abstract by Mr. G. Rigby.

MR. K. L. SMITH (Nottingham) remarked that the paper would have been more valuable if the authors had compared more than one sample of digitalis powder instead of examining all the tinctures prepared from one sample. He did not think that the paper proved that the chemical method gave results equivalent to the biological assay; it would be possible to think of other physical characteristics of the solution which, under the author's conditions, would have given the same potency ratio.

DR. G. E. FOSTER (Dartford) said that in his experience the Baljet reaction, unless used with a very sensitive absorptiometer, could be a snare and a delusion. The reagent, alkaline sodium picrate, was yellow and the colour formed was orange. Assays made with a visual colorimeter were unreliable owing to the inability of the eye to match colours of that nature. Digitalis was used therapeutically to slow down the heart but pharmacologically it exerted two effects: one was a cardiotonic effect which might be measured by the minimum dose required to slow the heart in a guinea-pig assay, and the other was the cardiotoxic effect which would stop the heart and kill the animal. Were these two pharmacological properties comparable? If the cardiotoxic effect was measured, was that a measure of the cardiotonic effect which was used clinically?

In some recent Japanese work, a glycoside of an acidic character had been isolated, and in that work it had been suggested that the cardiotonic and cardiotoxic principles were not the same. At this stage, it would be extremely dangerous for anybody to attempt to base the biological standardisation on the chemical assay. There was another difficulty with the biological assay of digitalis preparations. The international standard was a sample of dry powdered digitalis leaf. That was sometimes used in order to standardise such preparations as digitoxin. The substance chosen for the standard was not of the same nature as the substance under test. It would be better, if one were going to standardise a preparation of digitoxin, to use as standard a purified preparation of digitoxin as was done in the U.S. Pharmacopœia.

MR. H. S. GRAINGER (London) commented on the altered use of digitalis tablets in recent years. Clinicians had expressed the opinion that with the digitalis tablets now in use it was possible to achieve the desired clinical effect with a smaller dose, about half the previous requirement. This might be due to improved methods of preservation or of tableting and it would be interesting to know whether any alterations had been made in the criteria of the analysts in doing these assays.

MR. C. J. EASTLAND (London) asked whether the authors had carried out parallel assays on aged tinctures made from the same batch of leaf. He had examined a very large number of samples of the chloroform-soluble glycosides, which were a variable mixture of digitoxin, digitalin and some of the aglycones, and when a chemical assay was applied to such samples, there was a great variation in the ratio of these results to those obtained biologically. In a series of 14 samples, the ratio varied from 1 : 4 to 5. This was quite easy to understand if it was realised that the Baljet reaction depended upon the unsaturated lactone grouping in the glycoside molecule. If hydrolysis occurred to the extent of splitting up the sugar moiety, there would still be presumably the intact saturated lactone ring and that would still give the reaction. In a tincture there would be the aglycone present in the same proportion as in the original glycoside, but in isolated glycosides a good deal of the sugar fraction would have been eliminated and there would be a much higher proportion of the aglycone. If digitoxin were hydrolysed to give the aglycone and the aglycone were tested by the method in question, one would get a colorimetric assay roughly twice that given by the original glycoside. As ageing occurred, one would get an increasing quantity of aglycones, and he would like to ask whether, in the opinion of pharmacologists, such a tincture, though giving an assay figure corresponding to that of the freshly-made tincture, would have the same therapeutic effect.

MR. H. DEANE (Sudbury) said that there was not much evidence that the therapeutic activity of digitalis was closely proportional to the frog-killing power, and the different samples of digitalis did not produce parallel results when frogs, cats or guinea-pigs were used. He thought that everybody who had had to have physiological tests done would be

pleased if they could use a chemical method instead. The Pharmacopœia laid down limits of 7·6 and 12·4 and there were very few chemical tests which could not give closer results than that. They should be as close to the therapeutic effect as the biological method.

MR. G. RIGBY, in reply, said that the use of tinctures prepared from different samples of digitalis leaf was the next step in their programme. They must also attempt to separate the glycosides digitoxin and gitoxin in order that an assay of the content of each could be made in any particular tincture or sample of digitalis leaf. It was not claimed that the colorimetric assay would give satisfactory results if it were used for tinctures prepared from different samples of leaf.

For estimating the intensity of the orange colour produced in the Baljet reaction they had used the Spekker absorptiometer with the blue filters supplied with the instrument. The results were quite reasonable, with a standard deviation of 2 to 8 per cent. The fact that the biological assay depended for its end-point on the death of the animal and thus differed from the therapeutic object, was common to many such assays. However, it was the most suitable means available at the moment for assaying digitalis preparations. With regard to the cardiotonic and cardiotoxic effects he believed that it had been suggested that the adsorption of large amounts of digitoxin on heart or skeletal muscle and the various tissues of the body was irreversible while the adsorption of aglycones and of small amounts of digitoxin was reversible, or partially so, and that this irreversible adsorption led to accumulation of the drug which eventually might lead to the cardiotoxic effect. The international standard powder was of no use in making a comparison of a particular glycoside, but pure samples of digitoxin were available and one would, he imagined, compare an unknown sample of digitoxin with a pure one. As yet he had only assayed tinctures. He could think of no reason why the potency of digitalis tablets should be increased as reported by Mr. Grainger. A good deal of work had been done on the ageing of tinctures, and he thought that there was agreement that a tincture made with absolute alcohol was much more stable than one made with a weaker alcohol. This was possibly connected with the hydrolysis of the glycoside to the genin and aglycone. Such hydrolysis would not occur to any extent in a tincture prepared with absolute alcohol.

PROFESSOR H. BRINDLE, who also replied, said that the work described in the paper had been undertaken because in the American literature it was reported that the Baljet reaction gave a fairly accurate figure with regard to the activity of digitalis preparations when compared with biological methods. There was no satisfactory account from English workers and he felt it desirable that some work on the problem should be done in this country. Only one sample of digitalis leaf had been examined as it was thought better to examine the tinctures from this sample of digitalis thoroughly before proceeding with other studies. Over 1000 frogs had been used and the work had occupied about two years.

A POLAROGRAPHIC INVESTIGATION OF THE REDOX CHARACTERS OF THE AMINOACRIDINES, CONSIDERED IN RELATION TO ANTIBACTERIAL ACTION

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A study of the extensive literature dealing with the antibacterial drugs of the acridine series reveals three main lines of approach to the problem of their mode of action. Firstly, a large number of derivatives of acridine have been synthesised, and activity correlated with structure. Secondly, a number of physico-chemical properties of many members of the series have been determined and related to biological action; most of the knowledge accumulated in these two fields of investigation is due to the work of Linnell, Albert and their collaborators. Thirdly, the problem of biological activity has been partially elucidated through a study of bacterial nutrition and enzyme chemistry by McIlwain[1], Quastel and Wheatley[2], and Dickens[3]. Hinshelwood[4] and his collaborators have contributed important studies on the effect of some acridine derivatives on the lag-phase of bacterial cultures, an important feature of this work being the production of drug-resistant strains of bacteria by a process of training.

Perhaps the most widely accepted theory of acridine action is the basicity theory of Albert and his collaborators, who have shown that the most active members of the series are well ionised at *p*H 7, this condition facilitating attachment of the inhibitor to the surface of an enzyme protein. This theory does not account for the appearance of bacteriostatic activity within the series as a whole; as far back as 1922, it was recognised through the work of Browning *et al.*[5] that the intact acridine nucleus was necessary for full activity, and possessed some property not shared by the related heterocyclic compounds pyridine, quinoline, and phenazine. This conclusion was later supported by Berry[6], who found some acridanes to be quite ineffective as bacteriostatics, and also by Albert, Francis, Garrod and Linnell[7], who found acridone derivatives to be devoid of activity. More recently, Albert[8] has attributed the outstanding biological activity of the acridines as a whole to the flatness of the acridine molecule, which facilitates adsorption at an enzyme surface.

Following the work of McIlwain[1], who concluded that the acridines act by displacing a hydrogen carrier from the active centres of an enzyme, Breyer, Buchanan and Duewell[9] attempted to correlate the activities of a number of acridines with their reduction potentials, measured polarographically, and suggested a direct relationship between the two.

Certain anomalies were apparent in the polarographic results of these workers, who, while reaching the general conclusion that the acridines were reduced at the dropping mercury electrode in two one-electron

stages, described a three-stage reduction in the cases of acridine and 4-aminoacridine. In the former case, the height of the first step was found to be independent of concentration for concentrations between 10^{-4}M and 10^{-3}M. From this behaviour, it was deduced that acridine exists only as a dimer at concentrations higher than 10^{-4}M. This conclusion appears doubtful, for the mass law would predict some non-linear relationship between concentration and step height. The second step of reduction was found by Breyer *et al.* (*loc. cit.*) to disappear at concentrations below 10^{-4}M. To explain the peculiar behaviour of acridine and 4-aminoacridine, the formation of a compound between oxidant and the free radical product of the first reduction step was suggested.

The present series of polarographic studies was commenced firstly in order to elucidate the above anomalous behaviour of acridine derivatives, and, secondly, to attempt to find some redox property of acridine and its derivatives not shared by pyridine, quinoline, and phenazine, which might account for the activity of the former compounds and the comparative inactivity of the latter. The polarographic behaviour of acridine has already been described (Kaye and Stonehill[10]). In the present paper, the behaviour of some of the aminocridines at the dropping mercury electrode is described, and the biological significance of the results discussed.

Experimental

A manually operated polarograph was employed. The cell was a modified form of one described by Kolthoff and Lingane[11], and was constructed from a B40 Pyrex glass joint. The capillary constants in 50 per cent. alcoholic buffer solution were as follows: $m=0{\cdot}916$ mg.sec^{-1}, $t=4{\cdot}2$ sec. (with no applied potential). All dropping mercury electrode potentials were measured against an immersion type saturated calomel electrode, using standard potentiometric procedure. Current was measured by means of a calibrated damped Cambridge " Spot " Galvanometer. All experiments were carried out at 25°C. Cell solutions were de-oxygenated by passing cylinder nitrogen, purified by passage through a series of gas washing bottles containing alkaline hydrosulphite solution. The potential of the dropping mercury electrode was adjusted by a tapped resistance network forming a potential divider, and could be increased in steps of 10 mV., although steps of 20 mV. were usually employed.

For studying the current-time relationship during the life of a single mercury drop, an Ultrascope Mark I cathode ray oscilloscope was employed, in conjunction with an external time-base circuit giving a traversing time of about 5 seconds. This time-base, formed by charging a 10 mfd. condenser through a 1 megohm resistance from a 450-volt D.C. supply was not linear, but was found adequate for the purpose. The cell current was amplified by means of a D.C. coupled amplifier employing two EF50 valves, constructed in the laboratory. The output from the amplifier was fed to the Y plates of the cathode-ray tube.

Sörensen's buffer solutions served as supporting electrolyte. In many cases, 50 per cent. of alcohol was present in the cell solutions. The *p*H values of these alcohol-buffer mixtures were determined by means of a hydrogen electrode which could be inserted into the cell in place of the dropping mercury electrode.

Of the materials used, 5-aminoacridine and 2:7- and 2:8-diaminoacridine, in the form of their salts, were purified by repeated crystallisation from water. Very small amounts of the remaining mono-aminoacridines were available in a pure state, and these were not further purified.

Results and Discussion

All the mono-aminoacridines, and also 2:7- and 2:8-diaminoacridine in aqueous solution yielded anomalous polarographic waves, multi-step reductions being observed over most of the *p*H scale. The anomalies were most marked in the case of the mono-amino-acridines. Examples of the waves obtained are shown in Figures 1 to 3. Two main reduction waves were discernible, the complicating features being, in the case of the mono-aminoacridines, a fore-wave on the first main reduction step, and additionally, in the case of 1- and 2-aminoacridine, an after-wave on the second step. During the recording of the waves the galvanometer oscillations were observed to proceed in a very jerky manner, especially over the potential range corresponding to the fore-wave. Similar observations were previously made during the reduction of acridine (Kaye and Stonehill, *loc cit.*) and were found to indicate adsorption of electro-active material on the surface of the mercury drop, this being the cause of the anomalous reduction waves of acridine. It seemed probable that the multiple waves of the aminoacridines were also due to the same cause. To test this supposition, the current-time relations during the life of a mercury drop were studied oscillographically during the electro-reduction of 2:8-diaminoacridine. The results are shown in Figure 4. The horizontal time scale may be judged from the 50 cycle A.C. ripple superimposed on the tracings. The current-scale is indicated on the first tracing. Tracings A to F show the current-time relationship over the potential region occupied by the after-wave on the first reduction step; these tracings should be studied in conjunction with the correspondingly lettered polarographic wave shown in Figure 3. Tracings C, D and E

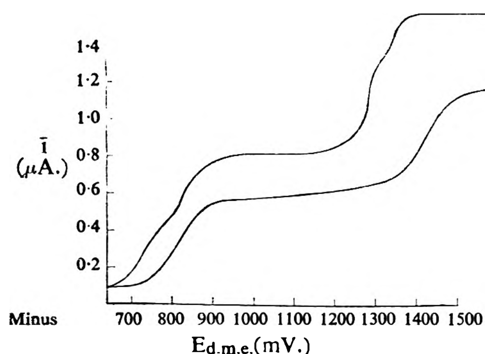


Fig. 1. Polarograms of 2-aminoacridine (4×10^{-4} M); upper curve, *p*H 7·38, without alcohol—lower curve, *p*H 8·29, with 50 per cent. alcohol.

show that after the detachment of the mercury drop, the current rises with abnormal rapidity to a small maximum value, marked on the tracings by a cross. Since the current flowing through the cell is composed of the electrons taken up by oxidant, it appears from these current maxima that the supply of oxidant to the drop surface is being hastened by marked adsorption on the mercury. The slight fall of current following the maxima is probably due to a temporary depletion of oxidant in the solution surrounding the drop; when this is made good by diffusion from the main bulk of liquid the current once more increases, as shown in the oscilloscope tracings.

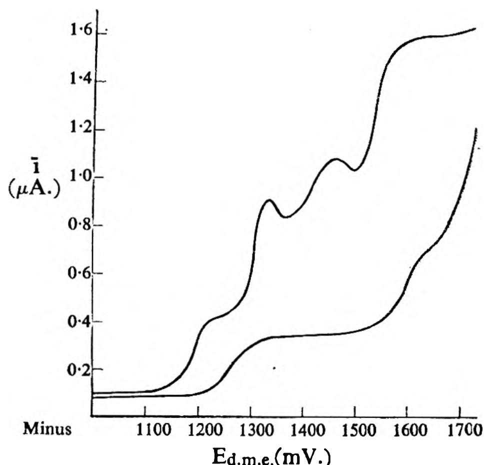


FIG. 2. Polarograms of 5-aminoacridine 2 × 10^{-4} M); upper curve, pH 9·07 without alcohol—lower curve, pH 10·39 with 50 per cent. alcohol.

Adsorption of oxidant at the dropping mercury electrode is in keeping with the presence of an after-wave on the current-potential curve, according to the theory of Brdička[12]. Treating the dropping mercury electrode as an inert-metal redox electrode, the electrode potential will be given by

$$E = E^{01} + \frac{RT}{nF} \ln. \frac{a_{ox}}{a_{red}} \quad (1)$$

where a_{ox} and a_{red} are the activities of oxidised and reduced forms of electro-active material in the interface boundary layer, near the mercury drop, n the number of electrons involved, R the gas constant, T the absolute temperature, F the faraday, and E^{01} the standard redox potential at a given pH. The presence of an appreciable amount of the oxidant in the layer in the adsorbed state will result in a lowering of the value of a_{ox}, and consequently a more negative value for E, and a displacement of the polarographic wave to the right. This displacement may be seen in Figure 3. Significantly, the abnormal current time relationships shown in Figure 4 occurred only over the potential range occupied by the after-wave.

It was shown by Kaye and Stonehill (*loc. cit.*) in a polarographic study of acridine that the inclusion of upwards of 50 per cent. of alcohol in the supporting electrolyte would render acridine less lyophobic and thus prevent adsorption of electro-active material on the mercury drop. It was therefore decided to study the effect of increasing alcohol concentra-

tion on the adsorption of 2:8-diaminoacridine at the dropping mercury electrode. The current-time relationships were studied during the reduction of this compound in buffer solutions containing 20, 50 and 60 per cent. of alcohol, the potential of the electrode being maintained at about −1050mV vs. S.C.E., corresponding to the crest of the after-wave. An alcohol concentration of 20 per cent. was quite ineffective in preventing adsorption (Tracing G, Figure 4). Increasing the alcohol content to 50 per cent. greatly modified the current peak (tracing H) and 60 per cent. of alcohol almost completely eliminated it. The inclusion of 50 per cent. alcohol, though not completely eliminating adsorption, permitted a normal polarographic wave to be obtained, shown in Figure 3 along with the anomalous wave obtained in aqueous solution. The displacement of the entire polarogram to more negative potentials in

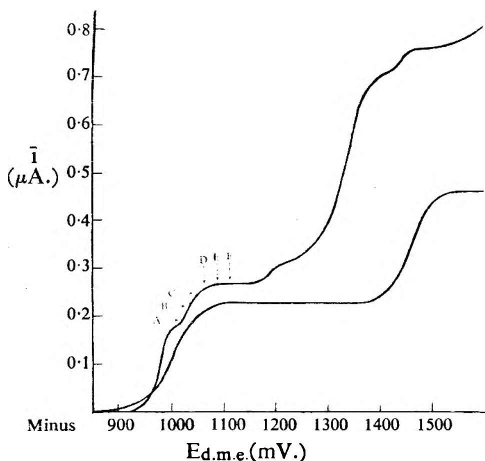


FIG. 3. Polarograms of 2:8-diaminoacridine (2×10^{-4} M); upper curve, pH 7·58 without alcohol—lower curve, pH 8·29 with 50 per cent. alcohol. Letters A to F correspond to Figure 4 tracings A to F.

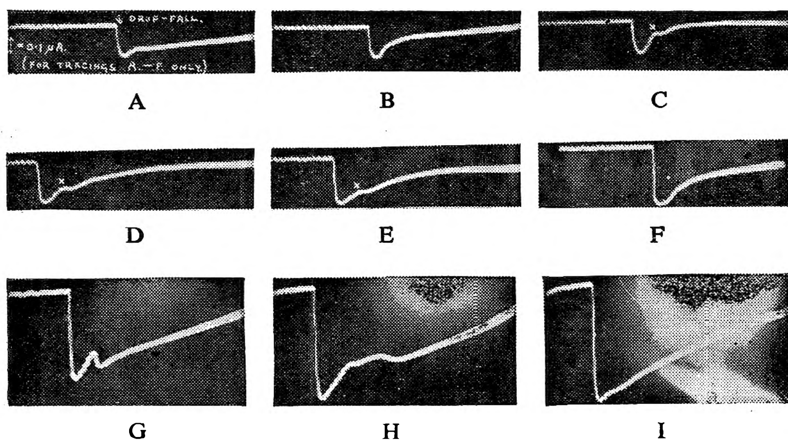


FIG. 4. Oscillograms of 2:8-diaminoacridine. Tracings A to F show the current-time relationship over the potential region occupied by the after-wave on the first reduction step. The white cross on C, D and E shows the small maximum attained with abnormal rapidity after the detachment of the mercury drop. G, H, and I show effect of addition of alcohol in eliminating current peak due to adsorption of compound on the mercury drop. Higher amplification was used for G, H and I

alcoholic solution is due partly to the increased *p*H of the solution, which was found to be 8·23 compared with 7·38 for the purely aqueous buffer.

The polarographic behaviour of 2:7-diaminoacridine was similar to that of 2:8-diaminoacridine, the after-wave on the first reduction step taking the form of a curious flattening of the crest of the main wave. Oscillographic study confirmed the adsorption of electro-active material at the dropping mercury electrode. In 50 per cent. alcoholic solution a normal polarogram was obtained.

The anomalies due to adsorption at the dropping mercury electrode were more pronounced in the case of the monoaminoacridines than in the case of 2:7- and 2:8-diaminoacridine, and consisted principally of a fore-wave on the first reduction step, and additionally, in the case of 1- and 2-aminoacridine, an after-wave on the second reduction step. Equation (1) shows that these anomalies may be explained by adsorption of the product of the first stage of reduction on the mercury drop. The monoaminoacridines were not fully studied oscillographically because of the small amounts available; a limited investigation showed a highly abnormal current-time relationship during the life of a single mercury drop. The use of 50 per cent. alcoholic supporting electrolyte enabled normal polarograms to be obtained, shown along with the anomalous waves found in aqueous solutions in Figures 1 and 2.

It is clear from the above evidence that the explanations offered by Breyer, Buchanan and Duewell[9] for the peculiar behaviour of the acridines at the dropping mercury electrode are not acceptable; evidently the three reduction steps described by these workers were not normal polarographic waves at all, but waves distorted by adsorption of electro-active material at the electrode. Breyer, Buchanan and Duewell reported lack of proportionality between step height and concentration in the case of 4-aminoacridine. The present investigations show that when adsorption is eliminated by the use of 50 per cent. alcoholic base solution there is good proportionality between the heights of both reduction steps and concentration.

The study of 5-aminoacridine was rendered more difficult by the occurrence of maxima (Figure 2). These were eliminated by working in 50 per cent. alcoholic base solution with the addition of 0·01 per cent. of methylcellulose, and by limiting the concentration of 5-aminoacridine to 2×10^{-4}M.

Breyer, Buchanan and Duewell (*loc. cit.*) reported the aminoacridines to be reduced at the dropping mercury electrode in two one-electron stages, calculated by means of the Ilkovic equation. It is possible that some of these results were invalidated by adsorption of electro-active material at the electrode, such calculations being valid only when the supply of the former to the latter is controlled by diffusion alone, which clearly is not the case for the acridines in aqueous solution. It therefore seemed desirable to recalculate the number of electrons involved (n) for the aminoacridines in alcoholic solution, in which adsorption does not

occur sufficiently to distort the reduction waves. These re-calculated values for *p*H 8·29 are given in Table I.

TABLE I

Compound	Concentration (millimols. per litre)	First step		Second step	
		$\bar{i}_d$ (μA.)	n	$\bar{i}_d$ (μA.)	n
1-aminoacridine	0·4	0·53	1·09	0·54	1·13
2 aminoacridine	0·4	0·49	1·00	0·45	0·94
3-aminoacridine	0·4	0·48	0·98	0·42	0·88
4-aminoacridine	0·2	0·22	0·90	0·25	1·04
4-aminoacridine	0·4	0·43	0·88	0·48	1·00
5-aminoacridine ... ... (*p*H 10·39)	0·2	0·24	0·99	0·28	1·17
2 :7-diaminoacridine	0·2	0·24	0·98	0·25	1·04
2 : 7-diaminoacridine	0·4	0·46	0·94	0·48	1·00
2 : 8-diaminoacridine	0·2	0·22	0·90	0·22	0·92
2 : 8-diaminoacridine	0·4	0·44	0·90	0·46	0·96

In these calculations it was necessary to have the value of the diffusion coefficient D, in 50 per cent. aqueous alcohol. The value of D for the aminoacridines at 25°C. was calculated from the value of D at 0°C. and the temperature coefficient as given by Breyer, Buchanan and Duewell (*loc. cit.*). The value of D in 50 per cent. alcohol was then calculated by analogy with the results of Gill[13], who found that D decreased by 38 ± 2 per cent. at 25°C. on adding 50 per cent. alcohol to solutions of anthraquinone 1- and 2-sulphonic acids, and also for some hydroxyanthraquinones. This procedure was supported by the more recent results of Shreve and Markham[14], who reported D for *p*-nitroaniline to be reduced by about 35 per cent. by alcohol concentrations of 46 to 55 per cent. The calculated value of D for the aminoacridines in 50 per cent. aqueous alcohol at 25°C. was $2{\cdot}92 \times 10^{-6}$. The results in Table I show that the aminoacridines, like acridine (Kaye and Stonehill, *loc cit.*), are reduced in two one-electron stages, and that the first product of reduction must be a free radical. This conclusion was supported by a polarographic study of phenazine, which, in strongly acid solution only, was found to be reduced in two well separated steps of heights almost equal to those of the acridines. The redox characteristics of phenazine, obtained by the polarographic method, were found to be very similar to those obtained for α-oxyphenazine by Michaelis, Hill and Schubert[15], who, by the potentiometric method, found this compound in acid solution to be reduced in two separate one-electron steps.

An outstanding feature of the polarographic reduction of the aminoacridines, and also acridine (Kaye and Stonehill, *loc. cit.*), is the wide separation of the two steps over the entire *p*H scale, a considerable increase of negative potential being required to convert the free radical to the fully reduced compound. This seems to imply unusual stability

of the free radical, the reason for which is not at present clear. In the case of acridine, free radical stability cannot be due to equivalent resonance, shown by Michaelis[16] to be of prime importance in stabilising semi-quinone type free radicals in solution.

In the case of reversible redox systems, the stability of the semi-quinone formed during reduction or oxidation is shown by the value of the semiquinone formation constant, k, given by

$$k = \frac{s^2}{r.t} \tag{2}$$

where s is equal to the concentration of semiquinone, r the concentration of reductant, and t that of oxidant in the half-reduced system. In such cases, k may be evaluated from the titration curve, using the equation

$$k = 10^{\frac{E_{ind}}{0 \cdot 059}} - 3 \times 10^{\frac{-E_{ind}}{0 \cdot 059}} \tag{3}$$

where E_{ind} is the index potential, i.e., the difference between the potentials corresponding to 25 per cent. and 50 per cent. reduction, or to 50 per cent. and 75 per cent. reduction. When the index potential is greater than 40 mV, lateral points of inflection appear on the titration curve, and it may then readily be seen that the reduction, or oxidation, is occurring in two univalent processes. For thermodynamically reversible systems, the polarographic wave is analogous to a reductive titration curve (Müller[17]). Treating the polarographic waves of the acridines in this way, the index potentials are given by

$$E_{ind} = \frac{E_2 - E_1}{2} \tag{4}$$

where E_1 and E_2 are the half-wave potentials of the first and second reduction steps.

On this basis, the semiquinone formation constants were found to be very large indeed. It is doubtful, however, if this procedure is valid, on account of the thermodynamic irreversibility of the electrode processes, deduced from the non-linear plot of E against log. $\frac{\bar{i}_d - \bar{i}}{\bar{i}}$ Nevertheless it is difficult to escape the general conclusion that during the electro reduction of the acridines free radical products of exceptional stability are formed.

In view of the variation of biological activity of the acridines with *p*H value reported by Albert, Rubbo, Goldacre, Davey and Stone[18], it was decided to investigate the variation of half-wave potentials with *p*H. Figures 5 and 6 show the E_1 and E_2 values for two of the amino-acridines in 50 per cent. alcoholic solution, plotted against *p*H. In interpreting these graphs, the following generalisations may first be made:

(1) The bends on the E_1/pH graph relate to dissociation constants

of either oxidant or free radical, while those on the E_2/pH graphs refer to dissociation constants of either free radical or reductant.

(2) Proceeding from left to right, bends consisting of a steepening denote dissociation constants belonging to the oxidant (in the case of the E_1/pH graphs), or to the free radical (in the case of the E_2/pH graphs). Bends consisting of a flattening denote dissociation constants belonging to the free radical (in the case of the E_1/pH graphs), or to the reductant (in the case of the E_2/pH graphs).

On this basis, equations, which would fit the experimental $E_{\frac{1}{2}}/pH$ curves were derived. It was found that the E_1/pH graphs for 2-, 4- and 5-aminoacridine and also for 2:7- and 2:8-diaminoacridine were described by the equation:

$$E_1 = E_0^1 + \frac{RT}{F} \ln \frac{K_1^S K_2^S h^+ + K_1^S h^{+2} + h^{+3}}{K^T + h^+} \quad (5)$$

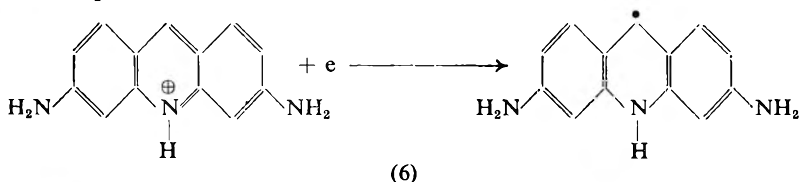
where E_1 is the half-wave potential of the first reduction wave, E_0^1 a constant, R the gas constant, F the faraday, K_1^S and K_2^S dissociation constants of the free radical, K^T that of the oxidant, and h^+ the concentration of hydrogen ions. This equation was tested by allotting values to K_1^S, K_2^S and K^T in accordance with the experimental values for 2:8-diaminoacridine and plotting the curve. Equation 5 takes no account of the bend occurring at pH 12 on the E_1/pH graphs for 2- and 5-aminoacridine, and 2:7- and 2:8-diaminoacridine. Since this bend occurred about the same point for all these compounds, it could not be due to dissociation constants of the oxidants; Albert and Goldacre[19] reported no pK_a values higher than 9·5 for these acridines. It was therefore concluded that this bend was due to some peculiarity of the polarographic technique, this view being strengthened by the unexpected finding of a similar bend for auramine at pH 12·5. It was significant that these bends coincided with the change from normal buffer solutions to sodium hydroxide solution as supporting electrolyte. Experiments showed that the effect was not due to increased ionic strength of the solutions.

The values of pK^T were found to agree well with the pK_a values determined electrometrically for 50 per cent. alcoholic solutions by Albert and Goldacre[19]. These values are compared in Table II.

TABLE II

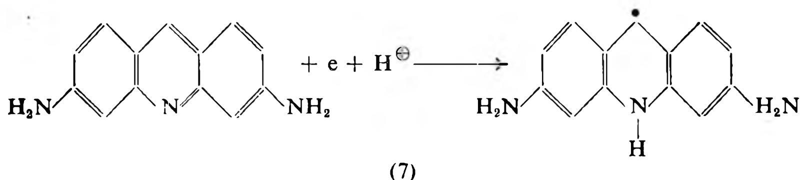
Compound	Polarographic pK_a values	pK_a values of Albert and Goldacre
2-aminoacridine	7·4	7·61
4-aminoacridine	5·6	5·5
5-aminoacridine	9·6	9·5
2 : 7-diaminoacridine	7·3	7·74
2 : 8-diaminoacridine	9·3	9·5
1-aminoacridine	3·8	3·59
3-aminoacridine	5·07	5·03

The most interesting feature of the first reduction step is its independence of hydrogen ion concentration over certain ranges of *p*H. This was found to be the case for 2-, 4- and 5-aminoacridine, and 2:7- and 2:8-diaminoacridine, but not for 1- and 3-aminoacridine. Evidently in these regions of *p*H reduction to the free radical stage requires the uptake of an electron only. Since the flat portions of the E_1/pH graphs always lie below the pK^T values, the oxidants will here be in the cationic form. Taking 2:8-diaminoacridine as an example, the electrode reaction can be represented as



(6)

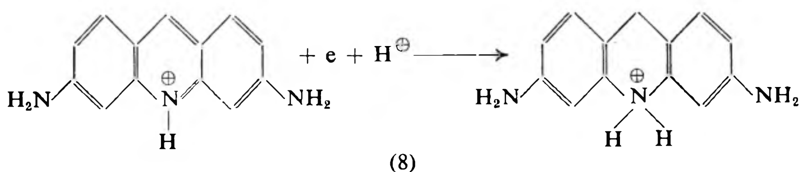
At *p*H values higher than pK^T a hydrogen ion must additionally be involved, and the electrode reaction will be



(7)

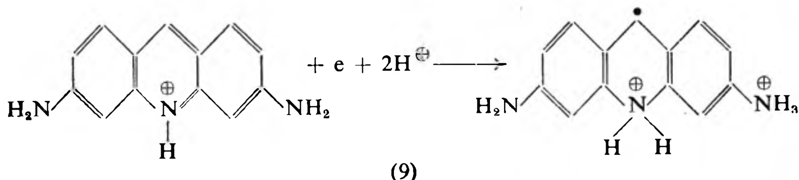
This reaction requires a slope of 59 mV for the E_1/pH graph at *p*H values higher than pK^T

At *p*H values between pK_2^S and pK_1^S, the electrode reaction may be represented as



(8)

again indicating a slope of 59mV for the E_1/pH graph over this range of *p*H. At *p*H below pK_1^S, the electrode reaction will be



(9)

resulting in a slope of 118mV for the E_1/pH graph at *p*H below pK_1^S.

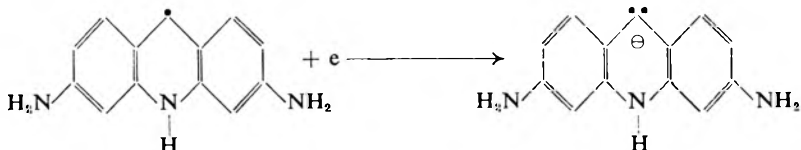
Inspection of experimental E_1/pH graphs shows that the slopes in some cases depart considerably from ideality, the best agreements with

theory being found in the case of 2:7-diaminoacridine. These non-ideal slopes may be attributed to the thermodynamic irreversibility of the electrode reactions.

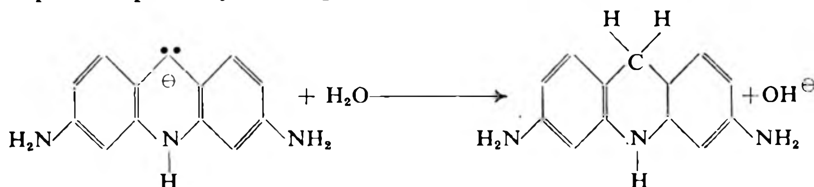
The form of the E_2/pH relationship was constant for all the aminoacridines studied, and was fitted by the equation

$$E_2 = E_0^1 + \frac{RT}{F} \ln \frac{K_1^R K_2^R + K_1^R h^+ + h^{+2}}{K_1^S K_2^S + K_1^S h^+ + h^{+2}} \tag{10}$$

where E_2 is the half-wave potential of the second reduction step, and K_1^R and K_2^R are dissociation constants of the reductant. The main interest in the E_2/pH graphs lies in their independence of hydrogen ion concentration in alkaline solution. Evidently the second reduction step here involves an electron only. Since the horizontal sections of these graphs commence in all cases at pH values higher than pK_2^S, the final reductant in this region of pH must be formed by the addition of an electron to the free radical product of equations (6) and (7). Consequently, the reductant must first appear as a negatively charged ion, according to the reaction



the product probably reacting with water:



The reduction mechanism is thus similar to that of the red form of phenolphthalein described by Kolthoff and Lehmicke[20]. These workers found this compound in alkaline solution to be reduced in two one-electron steps which were both independent of pH, and concluded that the final product of the two-stage reduction must first exist as a negatively charged ion having a lone pair of electrons on the central carbon atom.

The upward bend, shown by the dotted line, on the E_2/pH graphs was at first puzzling. Later, this was shown to be due to the increased ionic strength of the more alkaline base solutions; it was found that increasing the ionic strength of solutions of lower pH by the addition of potassium chloride shifted the half-wave potential of the second reduction step to considerably less negative values (cf. Burstein and Davidson[21]). The half-wave potentials of the first step were altered by only 2 or 3 millivolts by this treatment.

THE BIOLOGICAL SIGNIFICANCE OF THE RESULTS

The results of Browning *et al.*[5], Albert *et al.*[7] and Berry[6] all suggest that the intact acridine nucleus is essential for the development of full antibacterial activity in the acridine series. The complete inactivity of the acridanes (Berry, *loc. cit.*) and the acridones (Albert *et al., loc. cit.*) suggests that the activity of the acridines as a whole might be connected with their redox characters, a view which is supported by the polarographic investigation of Breyer, Buchanan and Duewell (*loc. cit.*), who found acridone to be reduced in one step only compared with the two step reductions of the biologically active acridines. These workers, however, interpreted their results differently, and suggested a direct relationship between bacteriostatic activity and the reduction potentials of the different members of the series, it being assumed that the difficultly reducible acridine derivative would take the place of a natural hydrogen carrier in one of the cell enzyme systems. This theory seems unlikely to be correct, for then all members of the series having a reduction potential more negative than a certain limiting value might be expected to be equally active as bacteriostatics, other factors being neglected. This was not found to be the case, however, and factors other than reduction potential must be more important in deciding activity. Further, Page and Robinson[22] showed that in general there is no direct relationship between bacteriostatic activity and redox potential.

Considering the biological activity of dyes as a whole, these appear to fall into two groups—those which behave as catalysts for cell respiration processes, and those acting as anticatalysts, or inhibitors of such processes. The first group includes thiazine and oxazine dyes, such as methylene blue, Capri blue, Nile blue and cresyl blue, which are able to function as hydrogen carriers in bacterial enzyme systems (Green, Stickland and Tarr[23]). Examples of naturally occurring substances playing the same rôle are coenzymes I and II, riboflavine, and pyocyanine. In the anticatalyst group of dyes would be placed the acridines and the triphenylmethane dyes, which were shown by Quastel and Wheatley[2] and by Dickens[3] to inhibit cell respiration processes.

It further appears that the activity of members of the second group can be neutralised by members of the first group. Thus, McIlwain[1] showed the bacteriostatic activity of euflavine and proflavine to be neutralised by methylene blue, cresyl blue, cozymase, riboflavine, pyocyanine and phenazine methosulphate, and that of crystal violet to be neutralised by pyocyanine and riboflavine. The resistance of *Ps. pyocyaneus* to the acridines reported by Berry[6] may thus be due to the pyocyanine content of this organism.

This evidence appears to indicate a competition between the respiratory catalysts on the one hand, and the inhibitors on the other hand, for places on the active centres of an enzyme protein, basicity being important not only for bacteriostatic activity, but also for respiration catalysis, as was demonstrated by Green, Stickland and Tarr[23].

In order to understand more completely the mechanism of these

opposing activities, it is helpful to review some of the theories proposed by Michaelis and also by Waters. Granick, Michaelis and Schubert[24] and also Michaelis[16] have proposed the hypothesis that bivalent organic oxidations and reductions can proceed only in univalent steps. Earlier, Michaelis and Smythe[25] had pointed out that the inertia of some organic compounds towards oxidation was due to their inability to form appreciable concentrations of semiquinone. Granick, Michaelis and Schubert[24] have also suggested that catalyst activity for oxidation processes, especially biological respiration, should be correlated with the ability of the catalyst to form semiquinones. Such catalysts would, of course, include the many substances functioning biologically as hydrogen carriers, some of which have been mentioned above. It is significant that many of these hydrogen carriers, both natural and artificial, are known to give rise to semiquinone radicals—pyocyanine (Friedham and Michaelis[26], Michaelis, Hill and Schubert[15]), riboflavine (Stern[27], Michaelis, Schubert and Smythe[28]), co-enzyme II (Adler, Hellstrom and Euler[29]), cozymase (Gutcho and Stewart[30]), methylene blue and other thiazines (Michaelis, Schubert and Granick[31,32]), and phenazine dyes (Kuhn and Wagner-Jauregg[33]).

The possible rôle of free radicals in biological respiration has been more clearly indicated by Waters[34], who has suggested mechanisms for cell dehydrogenations based on free radical chain reactions. The hydrogen carriers listed above would, of course, be able to enter into chain reactions of this sort, replacing the co-enzymes in the scheme proposed by Waters.

The results of the present investigations show that an outstanding property of the aminoacridines is their ability to form, during electroreduction, free radicals of unusual stability, indicated by the wide separation of the two steps of reduction. Such free radicals, unlike the much more labile radicals derived from the respiratory catalysts, would be unable to participate in the enzyme chain reactions proposed by Waters. On the contrary, it is conceivable that such chain processes, involving single electron exchanges, could be effectively terminated by the presence in the cell of a small concentration of acridine derivative through the ability of the latter to accept an electron, forming thereby a non-reactive free radical. The biological rôle of the inhibitor may thus be one of breaking chain reactions within the cell.

Albert, Rubbo, Goldacre, Davey and Stone[18] have shown that the most active members of the acridine series are 2- and 5-aminoacridine, and 2:7- and 2:8-diaminoacridine, while acridine, and 1-, 3- and 4-aminoacridine are very much poorer. Significantly, the present results show that the redox characters of the first group differ considerably from those of the second. These differences concern the first reduction step, i.e., the production of the free radical from oxidant. In the case of 2- and 5-aminoacridine, and 2:7- and 2:8-diaminoacridine, the first reduction step is independent of *p*H over the biologically important *p*H range of 7·0 to 7·4, reduction to the free radical stage requiring the uptake of

an electron only in this region of *p*H. The weaker members of the series, namely, acridine (Kaye and Stonehill, *loc. cit.*) and 1-, 3- and 4-aminoacridine do not show this behaviour, free radical formation requiring additionally the uptake of a hydrogen ion round about *p*H 7. In the case of 4-aminoacridine, the E_1/pH graph shows a horizontal portion, but not in this region of *p*H.

It may well be that the more biologically active members of the series owe their superiority to their ability to capture, and to hold fast in the form of a stable free radical, a single fugitive electron, which would otherwise be at liberty to participate in chain reactions profitable to the living cell. Electron transference is, after all, the essential feature of all oxidation—reduction processes.

Equation (5) shows that there are two requirements for the occurrence of a horizontal portion on the E_1/pH graph at about *p*H 7. These are

(1) the pK_a value of the oxidant must lie above *p*H 7.

(2) the pK_a value of the free radical must lie below *p*H 7.

The first requirement correlates well with the results of Albert *et al.*[16], who have shown that, for full activity, the pK_a value of the acridine derivative must lie above *p*H 7. In the light of the free radical theory of bacteriostatic action now proposed, the relationship between basicity and activity acquires an added significance.

If the superiority of 2- and 5-aminoacridine, and 2:7- and 2:8-diaminoacridine be due to their ability to form stable free radicals by accepting an electron only, then their activity would be expected to diminish as the *p*H of the medium falls below the *p*K value of the free radical, that is, into a region where free radical production requires a hydrogen ion in addition to an electron. Moreover, this behaviour would not be expected in the case of acridine and 1-, 3- and 4-aminoacridine, none of which exhibits a horizontal portion on the E_1/pH graphs in the region of *p*H 7. These conclusions are supported by data obtained by Albert, Rubbo, Goldacre, Davey and Stone (*loc. cit.*), who found, in the case of 5-aminoacridine and 2:7- and 2:8-diaminoacridine, a sharp decrease in activity with falling *p*H. The present investigation has shown that this decrease occurs approximately at the *p*K value of the free radical. Significantly, also, Albert *et al.* found the activity of 4-aminoacridine to be unaffected by falling *p*H.

A serious objection to the free radical theory of bacteriostatic action arises in the rather high negative potentials which would presumably be necessary for the production of the inhibitor free radical at the site of action. These potentials would be somewhere in the region of the E_1 values for *p*H 7 shown in Figures 5 and 6. (The potentials have been measured with reference to the saturated calomel electrode).

It may be doubted if such negative potentials would be encountered in the living bacterium cell, and it is not easy to settle this question. Attempts have been made to measure cell potential by immersing inert electrodes in their fluid cultures (Hewitt[35]). It is evident that such

FIG. 5. Variation of the half-wave potentials of 2 : 8-diaminoacridine (4×10^{-4} M) in 50 per cent. alcohol with pH

FIG. 6. Variation of the half-wave potentials of 3-aminoacridine (4×10^{-4} M) in 50 per cent. alcohol with pH

methods measure the potential of the culture medium on the cell surface rather than that of the cell interior. The use of redox indicators suffers from the same defect.

It is possible that attachment of the free radical form of the inhibitor to an enzyme protein will render E_1 less negative. The electrical potential required to convert oxidant to free radical depends on the different energy levels of these two forms; equation (1) shows that the existence of the free radical in an adsorbed state, of diminished activity, would result in a less negative reduction potential for the system.

It is not impossible, therefore, to meet the above objection; the free radical theory of dye stasis has received further support from the results of a polarographic investigation of the redox characters of triphenylmethane and diphenylmethane dyes, to be communicated in a further paper in this series.

Summary

1. The redox characters of some of the aminoacridines have been studied by the polarographic method. The anomalous behaviour of these compounds has been shown to be due to adsorption at the dropping mercury electrode, this conclusion being supported by oscillographic evidence. It has been shown that the use of alcoholic supporting electrolyte will eliminate adsorption and enable normal polarographic waves to be obtained.

2. The aminoacridines have been shown to undergo electro-reduction in two widely separated one-electron steps, the first product of reduction being a free radical, apparently of great stability.

3. A theory of dye bacteriostasis has been proposed based on the breaking of free radical chain reactions within the cell by the relatively stable free radical derived from the dye.

4. The variation of bacteriostatic activity within the series of aminoacridines has been related to the nature of the first step of reduction, which, for the most active members of the series, has been shown to require the uptake of an electron only at about *p*H 7. The variation of activity with *p*H has also been related to the nature of this first reduction step.

The author takes this opportunity of expressing his thanks to Dr. H. I. Stonehill and Dr. R. Gill for helpful discussions during the course of the work.

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References

1. McIlwain, *Biochem. J.*, 1941, **35**, 1311.
2. Quastel and Wheatley, *Biochem. J.*, 1931, **25**, 629.
3. Dickens, *Biochem. J.*, 1936, **30**, 1233.
4. Hinshelwood, *The Chemical Kinetics of the Bacterium Cell*, Oxford, Clarendon Press, 1946.
5. Browning, Cohen, Gaunt and Gulbransen, *Proc. roy. Soc.*, 1922, **93**, 329.

6. Berry, *Quart. J. Pharm. Pharmacol.*, 1941, **14,** 363.
7. Albert, Francis, Garrod and Linnell, *Brit. J. exp. Path.*, 1938, **19,** 41.
8. Albert, 11th Int. Congress of Pure and Applied Chemistry, July, 1947.
9. Breyer, Buchanan and Duewell, *J. chem. Soc.*, 1944, 360.
10. Kaye and Stonehill, *J. chem. Soc.*, awaiting publication.
11. Kolthoff and Lingane, *Polarography,* Interscience Publishers, N.Y., 1946.
12. Brdicka, *Ztschr. Elektrochem.*, 1942, **48,** 278.
13. Gill, Ph.D. Thesis, University of London, 1947.
14. Shreve and Markham, *J. Amer. chem. Soc.*, 1949, **71,** 2993.
15. Michaelis, Hill and Schubert, *Biochem. Z.*, 1939, **255,** 66.
16. Michaelis, *Ann. N.Y. Acad. Sci.*, 1940, **40,** 39.
17. Müller, *Ann. N.Y. Acad. Sci.*, 1940,**40,** 91.
18. Albert, Rubbo, Goldacre, Davey and Stone, *Brit. J. exp. Path.*, 1945, **26,** 160.
19. Albert and Goldacre, *J. chem. Soc.*, 1943, 454.
20. Kolthoff and Lehmicke, *J. Amer. chem. Soc.*, 1948, **70,** 1879.
21. Burstein and Davidson, *Trans. Electrochem. Soc.*, 1941, **80,** 175.
22. Page and Robinson, *Brit. J. exp. Path.*, 1943, **24,** 89.
23. Green, Stickland and Tarr, *Biochem. J.*, 1934, **28,** 1812.
24. Granick, Michaelis and Schubert, *Science,* 1939, Nov. 3.
25. Michaelis and Smythe, *Ann. Rev. Biochem.*, 1938.
26. Friedham and Michaelis, *J. biol. Chem.*, 1931, **91,** 355.
27. Stern, *Biochem. J.*, 1934, **28,** 949.
28. Michaelis, Schubert and Smythe, *J. biol. Chem.*, 1936, **116,** 587.
29. Adler, Hellstrom and Euler, *Hoppe-Seyl. Z.*, 1936, **242,** 225.
30. Gutcho and Stewart, *Anal. Chem.*, 1948, **20,** 1185.
31. Michaelis, Schubert and Granick, *J. Amer. chem. Soc.*, 1939, **61,** 1981.
32. Michaelis, Schubert and Granick, *J. Amer. chem. Soc.*, 1940, **62,** 204.
33. Kuhn and Wagner-Jauregg, *Ber. dtsch. chem. Ges.*, 1934, **67,** 361.
34. Waters, *Trans. Farad. Soc.*, 1943, **39,** 140.
35. Hewitt, Oxidation-Reduction Potentials in Bacteriology and Biochemistry (L.C.C.), 1948, 5th ed.

In the absence of the author this paper was taken as read and was not discussed.

REPORT OF A SYMPOSIUM ON FORMULARIES AND FORMULATION

A SYMPOSIUM SESSION was held on Friday, September 8, at 9.0 a.m., Mr. A. D. Powell, Chairman of the Conference, presided and the opening speakers were Professor J. P. Todd, Mr. H. S. Grainger, Miss M. A. Burr and Dr. A. Wilson.

THE THEORETICAL ASPECTS

Professor J. P. Todd said that his brief was the theoretical aspects of formulæ, formulation and formularies. He had no great practical acquaintance with formularies, unless one included in this category the British Pharmacopœia and the British Pharmaceutical Codex. In Scotland, they had little use for formularies and they foresaw possible danger in their development. It was possible that formularies might become so nationalised, and even internationalised, that there would be one book for all the formulæ in common use. This, of course, would not commit the prescriber to work exclusively on this national or international formulary, but it might produce a state of affairs where medicines were produced in some central institution, in London or even in Amsterdam, packed in cellophane containers and despatched all over Europe. He imagined that the effect of such a development on the future of pharmacy would be somewhat catastrophic.

It would be interesting to speculate on the growth and development of formulæ over the years, as possibly the devising of a formula is bound up with the development of civilisation itself. Formulæ—not necessarily pharmaceutical formulæ—must have occurred early in man's history. Formulæ made with the various drugs would develop in the course of time, so that we had at an early stage in our history collections of these formulæ, and notably the Pharmacopœias of London, Edinburgh, Dublin and, not least, Glasgow. The early pharmacopœias, however, were not formularies in the sense in which we use the term to-day, when the National Formulary tends to be the focus of attention and of great importance to every one of us. The early pharmacopœias were descriptions of preparations of the drugs then in use with standards and directions for making them. Pharmacopœias being, even from the earliest days, conservative in their views, there arose other works which tended to be more advanced and which could take greater liberties, due to the fact that they could, without running the risk of being taken seriously to task, introduce substances which might not prove themselves later on. Works of this kind tended also to become repositories for drugs which had been cast out of the pharmacopœia. In this way there was produced the second type of formulary, which has proved of great value over the years. The modern formulary tends to be more of a guide to the medical man; it suggests suitable compounds and admixtures of substances for the treatment of various ailments, and is less a book of directions to the dispenser. Some of the old books of many hundreds of years ago have survived the passing

of time and some of these and their formulæ are the old masterpieces of pharmacy. We should treat these old formulæ with the respect which is due to an old masterpiece; we should carefully preserve their history and hand it down to succeeding generations.

Professor Todd defined "formulation" as the art of presenting a substance in a form in which it best exhibits its characteristic properties. While every formula should have this as its primary object, other requirements were usually called for, such as safety in use, stability, convenience, elegance, or possibly the satisfying of some special demands due to special circumstances. It was to meet the demand for the secondary properties that the greatest ingenuity and skill were necessary. As a rule the primary objective was presented by the pharmacologist, whose work had followed the preparation by the chemist or other workers of the substance itself; it remained for the formulator to make the most of the primary objectives and enhance these if possible by meeting the secondary properties required. For example, the sulphonamides were first used orally, but they were quickly applied to the treatment of open wounds. The day of greasy bases for application to open wounds having passed, it was left to the formulators to incorporate these substances in oil-in-water creams which had the great advantage that they could be readily and painlessly removed in order to dress the wound; water was miscible with these creams and could remove them easily. The formulator, however, could rarely rest on his laurels, and it was found, when these creams were used on wounds, that a new bacterial flora developed in them which were resistant to sulphonamides, so that the preparations became of much less use. At that time, penicillin became available, and the formulator then had to devise ways and means of presenting penicillin in such preparations in such a way that it would exhibit its characteristic properties and not be destroyed in the process. The problem was thus temporarily solved, but in due course a bacterial flora developed which resisted penicillin.

Work of this kind implied co-operation between a number of specialists, one of whom was, of course, the formulator. In this connection, the skill and knowledge of the retail pharmacist were not used as they ought to be. There were rare exceptions to this, but the medical man and the local pharmacist rarely discussed a problem and attempted to solve it. The hospital pharmacist did this work regularly, and it could be one of the most important aspects of pharmacy in the future if it were developed.

The art of formulation has changed in character in the last few years. There is no doubt that there was plenty of art in old-time pharmacy, if there were but little science. Drugs were chiefly vegetable or inorganic in character, and many of them possessed romantic properties which inspired faith, but according to current medical opinion they had very little real action. The old formularies—purely local formularies, in the sense that they were old books of recipes treasured almost by every pharmacist—contained lists of drugs which read like a quotation from Keats: Irish Moss, Quince Seeds, Dragon's Blood, Almond Oil, Otto of Roses, and so on. The new remedies, the properties of which could be measured and the results submitted to statistical analysis, had altered

all this in the course of a lifetime, but there was still a great need for craftsmanship, which unfortunately was tending to die out.

There was a gap existing between the old-type formulation and that of the new remedies, and this gap required filling in. He could not believe that the pharmacy of the future, the new drugs which were coming on the market in such profusion, offered so little to the skilled pharmaceutical formulator as simply to be dissolved in sterile water and injected. There was ample evidence of the need for the skill of the pharmacologist and the bacteriologist, but there seemed very little left for the pharmacist. In the New Remedies Index issued by the Pharmaceutical Journal, 90 per cent. of the substances were complex organic chemicals but the pharmaceutical directions consisted of the words " Dissolve in sterile water." The remainder was made up of so much of a gram of the substance in the form of a tablet.

Now, what sort of pharmaceutical future does that offer? If the stark simplicity of the many preparations listed together with the compilation of international formularies and the making up of drugs at some central point were taken to represent the future of pharmacy, the outlook was not very bright. It was up to the pharmacist to develop a better future and no one was going to look after his interests—and incidentally those of the public—except the pharmacist himself.

One of the fields, however, which still offered great scope for ingenuity, and possible alternatives to parenteral administration, was the formulation of preparations, such as ointments and creams, balanced to allow of controlled absorption of the active principles. The sulphated fatty alcohols and substances like the wool alcohols, by careful blending with the older types of base, offered great scope for medication through the skin. The preparations in which water constituted the continuous phase have profoundly modified the properties of skin applications, and ointments and creams now offered possibilities for medication in this way which were not available with the older paraffinoid and lard types of base.

Far from being an impermeable barrier, the unbroken human skin afforded a ready means for the introduction of certain types of medicaments to the blood stream. If a solution of a water-soluble drug such as a sulphonamide were applied to the shaved skin of the rabbit, the sulphonamide could be detected and measured in the blood drawn from an ear vein inside 5 minutes. It might, in fact, be a dangerous procedure to apply preparations of this type over large areas. The application of a 10 per cent. sulphonamide cream to a large open wound had on one occasion proved fatal, although a 3 per cent. cream had occasioned no trouble. There were great opportunities and fields for research in discovering the possibilities of applying some of these drugs in this way. The sex hormones were already being prepared in this way, and there were commercial preparations on the market.

The introduction of tablets represented one of the advances made in the method of presenting substances for internal use. There was now the strict requirement that tablets should disintegrate in the human

stomach, and great credit should be given to those who had emphasised this point so thoroughly. Professor Todd said that many years ago he had been called in to a post-mortem examination where the colon of a patient was filled with tablets of quinine sulphate. These tablets dissolved in dilute sulphuric acid only with great difficulty.

The formulation of coatings which would prevent the disintegration of pills and tablets during the early stages of digestion was an interesting problem. When emetine bismuth iodide was first introduced for the oral treatment of amœbic dysentery there was a difficulty in that no matter how the emetine bismuth iodide was administered it was promptly returned. The properties of emetine were not sufficiently disguised by the insoluble compound which was used, and so various methods of coating the pills were attempted. Pills were used, as tablets were as yet unavailable. They were even coated, unsuccessfully, with wax. In this question of coating pills there were now definite signs of advance. When a property became measurable, it was then possible to make comparisons and to decide where progress had been made. Modern X-ray serial photography had solved some of the problems by enabling the formulator to follow the course of the pills through the alimentary system, thus enabling him to pick just that combination of solubles and insolubles which would allow the tablet or pills to dissolve at the right point.

Having found a suitable formula which possessed the necessary characters and had proved chemically suitable, the formulator was sometimes confronted with difficulties when he transferred the small to the large scale. Substances which remained in impalpable powders in pilot trials had the unhappy knack of crystallising out from large volumes during slow cooling, or preparations to whose success water was fatal were milled in wet mills. These call for readjustment and care but are seldom insuperable. There was still scope for research and ingenuity in the devising of better methods of presentation, and if the trials and troubles were great, satisfaction was equally great when success was achieved. There was no royal road to formulation; having defined the task the formulator must try over and over again until he reached his goal. This may mean fifty, sixty or a hundred attempts, but it was only by such painstaking effort that a good formula could be produced.

The Hospital Formulary

Mr. H. S. Grainger said that this was probably as appropriate a time as could be found for discussing the hospital formulary, especially with regard to its general use and status in the hospital, for two main reasons. The first was the appearance of the National Formulary, which was now familiar to every practising pharmacist, and which was intended to cover the needs both of general practitioners and of hospitals so far as the commoner medicaments were concerned. Its compilers had obviously envisaged the disappearance of the individual hospital pharmacopœias, because the preface states: "It is not suggested that hospitals should restrict the range of preparations in use, although it is hoped that duplication of formulæ of substantially the same composition will be

avoided, and that the scope of the formulary will obviate the need for individual hospital formularies for general medicine." The second reason was that changes in materia medica have considerably altered the status of the formulary within the hospital itself.

It might be useful to review the *raison d'être* of the hospital pharmacopœia or formulary, and it was convenient to refer to the formulary of Westminster Hospital. This publication originated in 1828, although there was in existence an earlier document, going back to 1721, which is referred to in the Minutes as a " pharmacopœia." This early document was just a list of the main drugs used in the hospital, which was compiled " to lessen the cost of medicines and (assist) the procuring them at best hand." It was used as a sort of tender form for the apothecaries of London in purchasing drugs for the hospital. From this list there gradually arose a compilation of formulæ which was eventually made into the hospital pharmacopœia of 1828. The primary purpose of the hospital pharmacopœia, therefore, was economy, and the pharmacopœia was an effective instrument to that end.

Until about the last two decades, almost the whole of the materia medica in use could be and were extemporaneously dispensed and were presented in the time-honoured form of draughts, mixtures, pills, ointments, lotions, plasters and so on. It was only recently that the great advances in microbiology, pharmacology and organic chemistry had produced the highly specific and complex substances the names of which, though perhaps less euphonious, were becoming more familiar than the old botanical names.

The hospital formulary contained all the remedies at that time deemed necessary and which were available in the hospital. Until very recent times, it was a rule at Westminster Hospital that no house officer was allowed to prescribe any drug which was not in the pharmacopœia, so that it was a comparatively simple matter to keep a firm grip on the drug bill. That is not possible now that most of the medicines used are chemical substances which come from the manufacturers' laboratories already formulated and ready for use.

One field, however, in which the hospital pharmacopœia could assist in the economical use of drugs was in presenting suitable alternative formulæ for compounded proprietary preparations. In many cases the hospital formula for such preparations was frank plagiarism, and no apology was made. The hospital pharmacist's criteria were clear. The first was that the product made in the hospital should be at least as efficacious; secondly, it must be as elegant as he could make it, though he did not claim in hospital practice and under hospital conditions to attain the degree of elegance which some proprietary manufacturers achieved; thirdly, it must be cheaper to the hospital than the proprietary preparation. This last point, of course, needed very careful consideration. One had to allow for the time and personnel available, and for other considerations such as the value of the overheads of one's department, and so on.

It was not always easy to produce an elegant preparation. and considerable effort was required in order to find suitable methods for hospital production. For example, the proprietary preparations of aluminium oxide gel were usually rather costly. The British Pharmaceutical Codex instructions did not produce an aluminium hydroxide gel which would necessarily be uniform from batch to batch, which had the correct thixotropic properties and was elegant and effective in use. When a suitable product had been devised, it was necessary to include it, for obvious reasons, in the hospital formulary. It was preferable not to use the proprietary name, or the words " substitute for " preceding the proprietary name.

The second function of the hospital pharmacopœia was that of a record of the preparations in use in the hospital and designed to meet the needs and predilections of the medical staff of that particular hospital. This is the chief reason why the National Formulary will never adequately supplant the hospital pharmacopœia.

On one occasion it had been necessary to devise an easily assimilated fluid preparation containing complete vitamin B requirements. This preparation was effective and subsequently it became the routine treatment for patients undergoing a certain treatment. It found its place in the formulary as Elixir Vitaminorum B (Westminster Hospital formula).

Wherever possible the National Formulary preparation was used, but there were some occasions when the hospital preparation was more acceptable and for this reason the hospital pharmacopœia was retained. It would, however, be republished under the title " Westminster Hospital Supplement to the National Formulary."

The third function of the hospital pharmacopœia was the part which it played in the training of medical students. The student's chief tool which enabled him to apply his theoretical knowledge in the ward was the hospital pharmacopœia, which contained those preparations which had the approval of his teachers and which by long practice had been accepted. The hospital pharmacopœia should be under constant revision by the medical staff and the pharmacist to see whether obsolescent preparations should be removed, or whether new preparations which had come into use should be included. In order to include these, the new formulary of the Westminster Hospital would be printed on pages which fit into a loose-leaf binder, for more ready revision. Most hospital formularies in the past have not been revised frequently enough.

As to nomenclature, indication of formula rather than function was to be preferred; but the medical student and the busy practitioner did not think primarily in terms of drugs but in terms of diagnosis, and he looked for a remedy the composition of which was secondary in his thoughts.

There was, however, a danger in that tendency being carried too far. Indeed, one senior physician went so far as to suggest that medical students should not be worried with quantitative memorisation of the doses, but that the latter should all be expressed in units—1 for a normal dose, 2 for a strong and 3 for an extra strong dose—and that the pharma-

cist should be saddled with the recollection of what that particular amount should be. For these reasons, we must stick to the nomenclature which is indicative of composition as an *aide memoire* to the student.

So far as hospital formularies were concerned, however, a place must be conceded to tradition. The traditional names should be added in parenthesis, in order to encourage and maintain interest in formulation among the medical staff. Where new preparations associated with the name of a particular medical man are introduced, tradition requires that within the bounds of the hospital that name should be given a place in the hospital pharmacopœia. As an example, there was a recently introduced preparation with the formula: glucose, 400 g.; arachis oil, 100 g.; powdered acacia, *q.s.;* water to 1000 ml. People do not remember the formula, but they have asked, "Do you remember that stuff that Dr. Bull uses?" and it has become known as "Bull's Mixture," so it is called "Emulsio Glucosi (Bull's Mixture)."

This sort of thing, however, can get out of hand, as in the case of a preparation which became known as "Mist. Euthanasia." This preparation contained morphine, hydrochloride, cocaine hydrochloride, alcohol and honey. It was intended for the alleviation of pain *in extremis,* and it should therefore be as pleasant as possible. This had been renamed "Haustus E."

In spite of the common point of view that medicines should be nasty, improvement could be made in some preparations at present in use. Certain brands of aspirin have been criticised for being prepared as confectionery instead of as a medicament, but the National Formulary or most hospital formularies cannot be accused of erring on this side. The formularies in use in Westminster Hospital for the last 80 years had undergone little change in the flavourings used—chloroform, peppermint, liquorice and occasionally tincture of orange. Considerable improvement could be made by experimenting with the new synthetic flavourings, and with new blends of some of the old preparations such as nutmeg, cloves, and oil of lime. Formulations of the kind discussed were still worthy of imaginative cosideration.

The Point of View of the Retailer

Miss M. A. Burr said that a retailer viewed formularies and formulation from many angles in a practical manner with a direct bearing on his own profession, business and training; his relationship with the prescriber and the patient; and, to-day, his dealings with the Ministry of Health. To-day the retailer was bound to regard the Ministry as his chief customer. The retailer bore in mind also the very important fact that formularies, past and present, were issued for the guidance of the practitioner.

The close relationship which exists between the retail pharmacist, the prescriber and the patient was very important and had provided the retail pharmacist with valuable practical knowledge. Much greater use could have been made of this knowledge in the revision of formularies.

Doctors were in the majority on the Committee which compiled

the 1929 Formulary and the present compiling Committee had a majority of pharmacists, but it was to be hoped that in the preparation of future Formularies there would be an increased number of retail pharmacists on the Committee.

When the formularies were considered which were in use prior to 1929 and even as far back as 1911, it was seen that that was a period of many formularies, formularies which caused a great deal of extra work and confusion in retail practice. There were similar titles used for different prescriptions. The National Formulary was the result of the progress which had been made, and it presented a uniform collection of prescriptions available in any part of the country.

Many retail pharmacists would like to see this uniformity carried a step further by the merger of the National Formulary with the British Pharmaceutical Codex. It could be seen, from the recent amendments to the National Formulary, that it was the desire of the compiling Committee to bring the National Formulary into line with the B.P.C. The formulary section of the B.P.C., for example, could be presented in a separate concise volume. The Danish Pharmacopœia, 1949, was published in three volumes, the third volume being devoted to formulæ.

In spite of the notice from the Ministry of Health, some doctors continued to use titles from the National War Formulary, and even from earlier formularies. They knew that on such occasions chemists might refer a prescription back to the doctor to be written in full, but retail pharmacists present would agree that this was no easy task. To encourage medical practitioners to use the National Formulary, and to stimulate a greater interest in and promote a better understanding of the preparations of both the National Formulary and the B.P.C., perhaps more publicity could be given to these preparations, for example at medical exhibitions.

Retail pharmacists would acknowledge the advantages gained by the use of these formularies in the State dispensing service. The National Formulary has a place in the National Health Service, but not an exclusive one. Freedom in prescribing should be encouraged and maintained. Some members had viewed some of the findings of the recent Cohen Report with concern. There may be a danger in over-emphasising the use of formularies.

In retail practice it was realised that one of the chief uses of formularies was economy. There were many aspects here that retail pharmacists had observed, the chief being the apparent lack of knowledge on the part of many practitioners of the National Formulary, particularly from the quantity point of view. The Committee compiling the Formulary had given much thought to this point and had stated the amounts to be dispensed, if not otherwise stated by the practitioner. To-day the wastage of both formulary and proprietary medicine must be enormous. The frequent dispensing of a pint of medicine to be taken in teaspoonful doses made one wonder what value the last ounce or so had. The same remarks apply to dressings. In regard to dosage, some consideration was long overdue. The pharmacist took the greatest care in preparing

the prescription, which was then administered in many cases in the most inaccurate manner. She was not referring to the patient who dispensed with any type of measure and drank straight from the bottle, or who doubled the dose and decreased the space of time between administrations, but to the domestic teaspoon. This could prove a most serious menace in the administration of children's medicines, and especially those containing certain potent drugs.

In the children's section of the Formulary there was great need for revision. The argument that medicine should not be too palatable might be a good one, but mothers would soon convince the Formulary Committee on this point. More use could be made of the vitamin syrups, blackcurrant and rose hip, where the ingredients would not destroy the vitamin C content. Colour was another point that needed consideration.

It would be advantageous if we had a uniform size and colour of tablets, and particularly, if coated, a standard colour for the coating. The proprietary list of the Formulary required amending at more frequent intervals and to be presented in a more useful way to the practitioner. With regard to the symbols for grains and grammes, a more explicit notice was needed than the general notice in the National Formulary. Preferably practitioners should use either grains or grammes and not both in writing a prescription.

To future revision Committees considering the presentation of the National Formulary the use of larger and clearer type in printing was recommended. It would also be helpful to have a thumb index, and paper of a different colour for the infants' section would be appreciated.

The Point of View of the Medical Practitioner

Dr. A. Wilson said that the year 1950 was one which seemed to encourage everyone to reflect on the changes which have taken place since the beginning of the present century. It was appropriate, therefore, that formularies and formulations should be discussed at the Conference, for this subject was intimately concerned with the practice of medicine and, in particular, might have a profound influence on the prescribing habits of the medical practitioner.

All would agree that remarkable advances had been made in the basic medical sciences and that these were reflected in the practice of medicine. There was ample evidence that custom and tradition had given place to a more certain and scientific approach to the diagnosis and prevention of disease. It would be reasonable, therefore, to expect comparable changes in the therapeutic methods which were employed to-day. It could not be denied that considerable advances had been made in therapy, but these were often overshadowed by inconsistencies and redundancies, many of which were evident in the drugs and preparations that were still used.

Much confusion arose from a failure to distinguish those drugs or preparations that had definite therapeutic and pharmacological activity from those relatively inert mixtures which, as Professor A. J. Clark has said, were administered from force of habit to gratify in an innocuous

manner the popular desire for a bottle of medicine. There was no doubt that the placebo had a very important part to play in therapeutic practice, but such preparations should be clearly recognised for what they were by all who prescribed them, and should not be confused with those which were designed to produce a specific therapeutic action.

In this connection, from the point of view of the medical practitioner, it was relevant to consider where he got his information about the nature and action of drugs. Apart from text-books and journals, the chief source was in pharmacopœias and similar publications. Professor Gunn, a man of considerable experience in these matters, said " Pharmacopœias can be taken to reflect, *conservatively*, the tendencies of pharmacological and therapeutic progress." This opinion might be modified with respect to modern pharmacopœias, but it was obvious to anyone who studied the history and development of these publications that they had never given full satisfaction to the therapeutic excursions of doctors and pharmacists. This was evident by the appearance at an early date of national formularies, codices and even extra pharmacopœias.

All these books were originally designed as books of reference for those engaged in prescribing or dispensing medicines, but most of them had been extended to meet the needs of analysts and others. They had thus become the medium for laying down standards not only for drugs which were active therapeutic agents but also for substances which were used in commerce and by the laity. This was as it should be, for society must be protected against the fraudulent seller of drugs, however potent or inert these drugs might be. It seemed strange, however, that, although such publications were undoubtedly the guardians of purity and constancy for drugs in common use, those in Britain did not appear to be upheld by any legal enactment.

Matters concerning the source, purity and methods of preparing drugs had become the domain of experts in these subjects, and the interest of the medical practitioner in pharmacopœias and allied publications was therefore mainly centred in those parts which dealt with preparations suitable for administration to his patients. This information, however, was not readily available. In our own British Pharmaceutical Codex, although it was contained in Part VI, there was to the medical practitioner who consults it a real risk of being submerged in a sea of infusions, decoctions and fluid extracts.

In this connection it was appropriate to comment on the question of fresh infusions. There were strict injunctions for the pharmacist to get these off his premises before they were more than 12 hours old. What use were they to a patient after that? The history of cinchona bark also made very fascinating reading, but was it necessary to keep this history alive by describing preparations of it in the B.P.C. which apparently were not incorporated in any of its formulæ?

Much the same criticisms applied to the U.S. National Formulary. The problem of selecting drugs and preparations was undoubtedly bound up with the major one of deciding the nature and scope of these publica-

tions. Too often it was apparent that in this matter decisions were based not so much on the effectiveness of the preparation as on the extent to which it was used.

There was an interesting account in the U.S. National Formulary of how this might be settled. An attempt was made to co-operate with the American Medical Association in preparing a National Formulary, but the American Pharmaceutical Association could reach no agreement with them. The medical men favoured a selection based on the therapeutic efficacy of the constituents, while the main committee preferred to continue a policy based on the extent of use. To this end a survey was made of all the drugs used for medicinal purposes, by determining the extent of use of these in prescriptions, in hospital and retail pharmacies and in drug stores. From the information gained it was decided to include in the National Formulary those items which were used in at least 20 per cent. of the drug stores or which were ingredients in at least one of every 10,000 prescriptions compounded in the United States. What a unique challenge to the advertising agencies!

This method of selection was by no means confined to the U.S.A., and it was practised in this country. From a legislative point of view this collected information was no doubt necessary to ensure that drugs which were commonly used conformed to standards of purity; it may well be that the B.P.C., like the U.S. National Formulary, should undertake this task and follow this policy.

The medical practitioner, however, looked for information where the facts were available in a ready and concise manner. He needed a formulary containing a reasonable selection of therapeutic agents, described in a manner suitable for simple prescribing and administration. The principle of selection should be based on therapeutic efficiency.

The nearest approach to such a publication was provided by the British National Formulary, 1949. This was the combined effort of the medical and pharmaceutical professions and was a commendable attempt to provide a collection of formulæ consistent with therapeutic usefulness and pharmaceutical skill. It contained the essential information about the active ingredients of drugs and their preparations and their official doses. To a limited extent it had been bold. It had excluded two drugs —one, the dangerous and deadly heroin; the other, the inert and innocuous bismuth carbonate. It was obvious, however, that selection even here was too often based on the extent of use rather than on efficacy. It was true that buchu no longer adorned the formulary and that the glycerophosphates were now debarred, but other equally useless and confusing preparations should also have been swept out. Was it necessary to have six bitter-tasting preparations, or was it that mixture of strychnine and iron was masquerading as a potent preparation of iron?

The subject of doses caused much confusion. In Britain there was a curious complex about doses. We were very timid in using adequate doses of drugs. Perhaps this attitude arose from the guidance given by

the B.P., with its range of doses the choice within which, it was careful to point out, must be left to the medical practitioner's own judgment. But why not give him more precise facts to enable him to exercise this? The U.S.P. definition of an average dose gave more guidance.

Let us examine briefly how the compilers of the National Formulary have exercised their judgment regarding the doses of ingredients. Amongst the preparations designed, presumably, for the symptomatic control of diarrhœa there was a mixture with chalk which contained an equivalent of 6 mg. of morphine in each dose, whilst another with kaolin had only 1 mg. of morphine. Were both preparations effective? To render the urine alkaline the maximum B.P. dose of potassium citrate had been used, but only the minimum dose of tincture of hyoscyamus was used in another, similar, preparation. In the mixtures of aspirin and of potassium bromide they had plumped for half the maximum dose. Perhaps this was an attempt to conform with extent of use, but surely the latter preparations were not consistent with the modern knowledge of bromide action?

Frequency of administration was an important factor concerned in building up or maintaining the desirable concentration of a drug in the tissues. The pharmacological principle of this in relation to the action of salicylates was too well known to be elaborated here. In the National Formulary there was a reliable and potent mixture of sodium salicylate. There was now a suggestion to reduce the content of salicylate because of the reported occurrence of dizziness in a few patients. To control these toxic effects, when they arise, all that was required was to direct the patient to take a smaller dose of his medicine. Why spoil an effective remedy when toxic reactions could be adequately controlled in a more rational manner?

The doses of drugs and preparations were described in the metric and imperial systems. The arguments for and against the retention of both could not be discussed here, but there should be a little more consistency in setting out the information. In the section dealing with tablets, the contents of the more recent drugs were given in the metric system, whilst those of the older drugs were given in the imperial system. This promoted confusion.

Forty years ago antitoxins, vaccines and sera were hailed as the therapeutic hope of the future. They found no place in the National Formulary. Could it be that these were no longer regarded as satisfactory therapeutic agents to be used by medical practitioners, or was it that they did not rightly fit into a formulary of drugs?

So much for the nature and content of the Formulary. Let us now consider the arrangement and manner of setting out the information contained in it. So far this had followed the familiar pattern whereby preparations were listed and described in alphabetical order. This might be a convenient system for the pharmacist, but the medical practitioner would derive more information, and that more quickly and easily, by

consulting a book in which preparations were arranged according to the bodily systems on which they predominantly exerted their effects.

Suppose, for example, the practitioner wished to control attacks of asthma in his patient. If he consulted the index of the National Formulary, there was no guidance under ephedrine. He must plough his way through all the possible Latin names of preparations that he knew, and this might range from elixirs on page 27 to tablets on page 79. It would be much more helpful and instructive for him if all the drugs and preparations acting predominantly on the respiratory system were collected together. He would then, probably for the first time, realise that there were available eight or nine effective preparations for the treatment of bronchial spasm.

A pharmacological classification of the constituent preparations would be a more satisfactory method of presenting the valuable information which was contained in the National Formulary. Too much attention should not be paid to the invention or retention of Latin names for preparations; it was much more important to ensure that the facts were readily available.

Dr. Wilson showed, by means of lantern slides, four prescriptions illustrating the tendency of some doctors to prescribe proprietary articles, and to include large numbers of ingredients in their medicines, and concluded: These are perhaps extreme examples of present-day prescribing, but they reflect the confusion which arises from a failure to appreciate just what drugs can and cannot do. Several factors may account for this state of affairs, and one of these may have its origin in the limited scope of the medical curriculum for the teaching of pharmacology and therapeutics; another may be in the flow of enthusiastic but totally unwarranted therapeutic reports from commercial agencies. I should like to suggest, however, that a formulary based on the principles which I have discussed would give immediate and practical guidance to the medical practitioner in designing and in implementing his therapeutic programme.

DISCUSSION

Dr. K. R. Capper (London) said that it had been suggested that the National Formulary and the formulary section of the British Pharmaceutical Codex should be merged. It should be borne in mind that most of the preparations in the National Formulary were in the British Pharmacopœia or the B.P.C.; out of about 500 preparations in the National Formulary, only about 30 were not in one of those books. The Codex had an important function in setting standards for these preparations and stating methods of assay. These standards were a protection to the pharmacist as well as to the public, because limits of tolerance were given which were based on a knowledge of the conditions in which these preparations were made up and of sampling errors, etc. If it were laid down that every preparation must contain 100 per cent. of what was supposed to be in it, few preparations could possibly comply. The Codex and the N.F. were complementary and should not be amalgamated.

He approved of various hospitals producing supplements to the N.F. in place of their own Pharmacopœias but preparations should not be called after the medical practitioners who were responsible for them. The only way of dealing with Dr. Wilson's suggestion about the therapeutic arrangement of the items would be to produce two formularies, one for the medical profession and one for the pharmacists. The Joint Formulary Committee were at work on the question of flavourings and the difficulties which existed, particularly with children's medicines, were well known.

DR. E. HÖST MADSEN (Denmark) remarked that there had been some criticism of international formularies. This kind of work proceeded very slowly, but it was worth while as, in the end, it was to everyone's gain and the results obtained in individual countries became the property of all countries.

Reference had been made to what should be the object of a pharmacopœia. In the U.S.A. it was intended to be a compilation of the most used formulæ. In Scandinavia they were of the opposite opinion; they thought it should not be a compilation but a guide on a national basis for medical men. They wanted to exclude all formulæ which were no longer regarded as rational and to give as quickly as possible new formulæ in a form which could be recommended. The highest responsibility in the country in medical circles was that of the Pharmacopœia Commission. He was very glad to hear Miss Burr mention the Danish Pharmacopœia, consisting of three volumes. There was also a fourth volume; it gave only shortened formulæ from the Pharmacopœia with indications of how they should be prepared, but with a brief account of their therapeutic uses. It was intended for doctors and could be carried in the pocket. Formerly they had had, as in this country, hospital formularies and formularies issued by the Pharmaceutical Society, but now these were published as one volume by the Pharmacopœia Commission and a new supplement was issued annually. He would recommend that consideration be given to producing a book similar to this fourth volume of the Danish Pharmacopœia.

MR. A. W. BULL (Nottingham) said he would like to take up the theme elaborated by Professor Todd that pharmacy was not accorded the recognition which it deserved as it was regarded as inferior to those sciences which together make up pharmacy in the broader sense of the word. The practising pharmacist could make a very important contribution to formulation. Often a formula could not represent both the therapeutic ideal and the pharmaceutical ideal so that the skill of the formulator then came into play in arriving at the best possible compromise.

In many instances necessary information and data could not be found in the literature, and the details had to be worked out or suitable formula produced by trial and error. An example of the difficulties arising in formulation and the way in which they are overcome would be found in some work done by his colleagues on strong eyedrops of sulphacetamide

B.P.C. The general preamble to the monographs on eyedrops in the Codex states that where possible they should be made approximately isotonic equivalent to a 0·9 per cent. solution of sodium chloride in water. They represented a pharmaceutical ideal but how far was it possible to go towards that ideal? First, was it necessary to have such a high concentration as 30 per cent. of sulphacetamide? The administration of such a high concentration at once stimulated a copious discharge of the lachrymal secretion, which would dilute the effective concentration of the preparation. What in fact was the actual working concentration following that administration? Again what were the tonicities of a solution of this substance of concentrations ranging from 0 to 30 per cent? A 30 per cent. solution was hypertonic. How did it compare with the pharmaceutical ideal of 0·9 per cent. solution of sodium chloride and what was the effect of concentration on the causation of pain to the patient? The boric acid contained in the B.P.C. formula was perhaps to neutralise the solution but the N.F. stated that this substance yielded a very soluble and nearly neutral sodium salt which could be applied to conjunctivae in concentrations of up to 30 per cent. These problems and questions arise from just one preparation and the same question might be asked about many others where it was necessary to compromise between pharmaceutical and therapeutic ideals.

MR. J. W. HADGRAFT (London) said that he would agree with Dr. Wilson on the need for two editions of the formulary. Many remedies remained in the B.P.C. simply because there was a demand for them, such as glycerophosphates which were now thought to be therapeutically inactive. Another problem was the increasing use of proprietary preparations. Now that there was a need for economy in the National Health Service care should be taken to avoid giving official or semi-official recognition to a combination of drugs solely on the ground that there was need for the provision of a less expensive non-proprietary formulation. He thought that Latin even in the writing of prescriptions should be abandoned. The flavouring agents available in the official books were too limited in their type and would appeal rather to the adult palate than to that of a child, and there was a definite need to make medicines more palatable for children. It was desirable to use one system of weights and measures. Not long ago the ludicrous position was reached that formulæ in the National Formulary were based on accurate imperial quantities while in the B.P.C. they were based on accurate metric quantities and accurate percentages, with the result that it was necessary to bring out a special addendum to the National Formulary in order to bring it into line with the B.P.C. He hoped that the metric system would be adopted.

MR. T. D. WHITTET (London) said that at University College Hospital it had been decided, for historical and traditional reasons, to keep their own pharmacopœia. As far as possible they complied with the N.F. and B.P.C. formulæ; in only a few cases did they make a serious alteration. One such case was a mixture which Dr. Wilson had mentioned, mixture of sodium salicylate. Recent research had shown that

many patients could tolerate sodium salicylate without the presence of sodium bicarbonate and, if they could, one got a higher blood level with a similar total dose of salicylate. To bring the hospital pharmacopœia up to date they issued what were known as "therapeutic notes." These comprised a small leaflet giving notes on the more important drugs and which fitted into a space in the back of the pharmacopœia. He would agree with Mr. Grainger in deprecating the outrageous names which were sometimes coined in hospitals. In addition to palatability, suitability and elegance were important properties. Nevertheless unpalatable mixtures are even welcomed by certain patients.

The problem of *p*H in galenicals and injections was very important and needed investigation. In the 1948 B.P. control of *p*H was more frequently applied as compared with the 1932 B.P. He thought it very important indeed to have the proposed new official formulæ tested out practically before use, and preferably stored for some time. There was a need for a laboratory, having a full-time staff, to carry out these investigations either under the aegis of the B.P.C. or the Pharmacopœia Commission. Of the newer solvents available he had found propylene glycol very useful. It was less toxic than glycerin and many substances were more stable in it than in aqueous or alcoholic solution. He had successfully used it for barbiturate elixirs and for injections. A suitable strength was 50 to 60 per cent., and 2 per cent. of benzyl alcohol was added to the injections in case the propylene glycol caused stinging. Ascorbic acid was soluble to about 8 per cent. and much more stable than in aqueous solutions. In this form ascorbic acid was very useful for adding to infants' foods. Calciferol was readily soluble in it and had recently been reported to be 2 or 3 times more active when administered in this way than when in an oily solution.

The sorbitan derivatives ("Crills" and "Tweens") were very useful in solubilising volatile and fixed oils. Some of them could not be taken internally but others were non-toxic. For example, clear aqueous preparations of vitamin A could be made by using polyoxyethylenesorbitan mono-oleate; they were completely miscible with water and could be made quite palatable. Such emulsions of vitamin A were also stated to be more effective than the oily solutions. Elegant preparations of all the common vitamins were easily prepared by use of propylene glycol and the sorbitan derivatives.

DR. G. E. FOSTER (Dartford) referred to Miss Burr's suggestion of the use of uniform die sizes for tablets. There was some danger in this as the different sizes were useful in differentiating between tablets, particularly those of similar nature. If there were official requirements introduced concerning the sizes of tablets he hoped different die sizes would be used to prevent error.

MR. F. H. OLIVER (Sunderland) said that the therapeutic action of any medicament was due to its pharmacological action and its psychological action, and he thought that the latter aspect of formulation was often forgotten. The ancillary substances which occurred in medicines often contributed largely to the psychological effect, especially in children.

By unsuitable medication it was possible to build up inhibitions which might last the child's lifetime. He welcomed the idea of an edition of the N.F. with the preparations grouped pharmacologically, as this might lead to less rubber stamp prescribing.

MR. V. REED (London) suggested that some formulæ contained far too many ingredients, and also stressed the need for making children's mixtures more palatable.

MR. W. R. HOWARD (Hornchurch) was concerned at the considerable waste of time and effort which went on after a great deal of time and effort had already been devoted by the manufacturer to the careful formulation of his products, a second investigation was conducted by the hospital pharmacist endeavouring to produce an equally elegant and stable preparation. Some of the problems of stability and elegance did not always seem to have received sufficient attention at the time that a formulary was prepared. Problems were met with in large-scale production which did not crop up in *ad hoc* compounding. He therefore suggested that where formulæ contained vehicles or ingredients which were themselves liable to variation (e.g., tragacanth or agar), some latitude should be allowed to the compounder in the use of these materials and the final result should then be controlled by standards laid down in the appropriate publication. The standardisation of size of tablets would inevitably raise the question of the standardisation of formulæ. At the moment, formulæ were very much the subject of art and, fruitful though the field might be for planning, no official body had yet tackled it. The problem would be fraught with many difficulties and would obviously be hedged about with considerations of ready distinguishability for the different doses and similar chemical compounds with markedly different properties.

MR. J. C. HANBURY (Ware) said that, while he agreed that various books of standards performed a most useful function, he thought that if there was any pressure from outside the medical profession to compel medical men to confine themselves to such books, then a grave disservice to the progress of medical science would have been done. He thought that the art of pharmacy was changing rather than disappearing. There was more art, and infinitely more science, in producing some of the modern preparations than some of the rigmaroles of the B.P. 1867 and the like. One had only to think of the problems, both scientific and artistic, in producing satisfactory stable injections of vitamin B_1, and riboflavine and vitamin products of that sort. There was endless scope for pharmaceutical ingenuity in the newer galenicals. The suggestion of a therapeutic index was most valuable. The rôle of the pharmacist in medical science was changing; he must now become more of a pharmacologist and must be trained to do more than merely carry out the wishes of the physician, though the physician must always have the last word. The pharmacist must thoroughly understand the drugs in common use and be in a position to advise the physician on their use and methods of preparation.

MR. A. R. G. CHAMINGS (Horsham) asked what was the criterion of a good drug and who was to decide upon it? It was certainly necessary that any formulary should have a sound pharmacological basis.

MR. R. W. GILLHAM (Leeds) said that non-adherence to standard formulæ in hospitals caused a great deal of unnecessary work. He was sure that slight differences in the doses of various medicines and ointments did not make the slightest difference in effect on the human body, because it was not a volumetric machine. Not everyone realised the problems of transferring small-scale formulations to manufacturing quantities.

PROFESSOR J. P. TODD, in reply, said he hoped his views on the National Formulary had not been misunderstood, but he thought they should guard against the day when all medicaments might have to be bought from a central store. He had not intended to say anything against the International Pharmacopœia. This was a totally different problem from the National Formulary, and he greatly valued and appreciated the work which had been done in connection with it.

MR. H. S. GRAINGER said that he would be very sorry if they were so pedantic as to destroy all sense of tradition and all interest on the part of the medical profession in hospital formularies. He agreed with Mr. Howard that much work was being done in the hospital dispensary which had already been done by manufacturing houses. Nevertheless, ways of keeping down expenses had to be investigated. He would hesitate to limit a practitioner to a particular dose of a drug and in spite of the inconvenience he was encouraged when he saw a young house officer or medical registrar exercising a little individuality and ingenuity in the writing of prescriptions.

MISS M. A. BURR thanked the speakers for being more or less in full agreement with what she had put forward. The co-ordination and extension of pharmaceutical research was very important indeed, and she felt that the Pharmaceutical Society could sponsor something in this connection, which would be of great use in all sections of pharmacy if it were brought into being in the near future.

DR. ANDREW WILSON said that they had been reviewing what had happened over the past 50 years, and he hoped planning for what might take place in the next 50. He hoped that they would go away feeling that the pharmacist must make it his business to know not only the nature and quality of a drug, but also something about how it acted.

THE CHAIRMAN thanked the opening speakers and all who had taken part in the discussion, and the meeting then terminated.

RESEARCH PAPERS

NUCLEAR DERIVATIVES OF 4:4'-DIAMINODIPHENYL SULPHONE

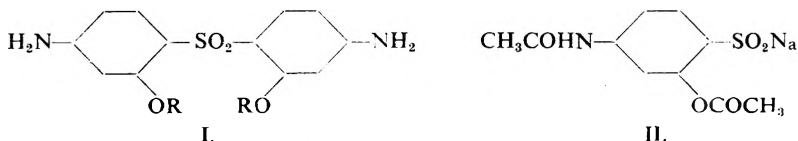
By W. H. Linnell and J. B. Stenlake

From the Pharmaceutical Chemistry Research Laboratories of the School of Pharmacy, University of London

Received October 12, 1950

Attempts to lower the toxicity of 4:4'-diaminodiphenyl sulphone have resulted in numerous modifications of its chemical structure. Few, however, of the important group of *N*-substituted derivatives are more active against *Mycobacterium tuberculosis* than the parent compound and there is some evidence that they are converted in the body into 4:4'-diaminodiphenyl sulphone[1]. Nuclear substituents, too, contribute little to the level of *in vitro* activity[2,3] and in many cases marked reductions have been reported. Two compounds, of interest because of their structural relationship to *p*-aminosalicylic acid, 4:4'-diamino-2-hydroxydiphenyl sulphone[2,4] and 4:4'-diamino-3-methoxydiphenyl sulphone[3], possessed respectively one quarter and twice the *in vitro* activity of the parent compound, but *in vivo* studies and toxicities of these compounds have not been recorded.

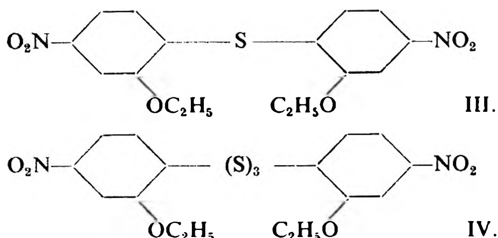
The established activity of *p*-aminosalicylic acid against *M. tuberculosis* and its low toxicity, coupled with the known biological oxidation of sulphanilamide to 4-amino-2-hydroxy-benzenesulphonamide which occurs in certain animals[5], prompted a further investigation of ortho hydroxylated substituents in the molecule of 4:4'-diaminodiphenyl sulphone. The synthesis of 4:4'-diamino-2:2'-diethoxydiphenyl sulphone (I, $R = OC_2H_5$) and 4:4'-diamino-2:2'-dihydroxydiphenyl sulphone (I, R = H) is now reported. Since the conclusion of this work the preparation of the latter compound has been described by Amstutz[6].



Attempts to condense sodium 4-acetylamino-2-acetoxy-benzenesulphinate (II) with 2-chloro-5-nitrophenol, according to the method described by Ferry, Buck and Baltzly[7] for the preparation of 4:4'-diaminodiphenyl sulphone, were unsuccessful. Under similar conditions *p*-bromonitrobenzene also failed to condense with II. Burton and Hu[8] have reported the failure of comparable reactions between various unspecified aryl iodides and sodium *p*-cyanobenzenesulphinate and, more recently, Peak and Williams[9] described unsuccessful attempts to obtain sulphones by the condensation of 2:4:6-trichlorobenzenesulphinate with diethylaminoethyl chloride.

The required compounds were obtained by the repeated oxidation of

2:2′-diethoxy-4:4′-dinitrodiphenyl sulphide (III) with an excess of potassium permanganate to yield the corresponding sulphone, followed by combined reduction and de-ethylation with hydriodic acid. The latter reagent, with suitable adjustment of the reaction conditions, gave either one or the other of the required products, 4:4′-diamino-2:2′-diethoxydiphenyl sulphone or 4:4′-diamino-2:2′-dihydroxydiphenyl sulphone.



Condensation of 4-chloro-3-ethoxynitrobenzene with sodium sulphide by the method of Hodgson and Dodson[10] failed to yield the monosulphide III, and 2-chloro-5-nitrophenol was isolated in 73 per cent. yield, together with a small quantity of a second product, molecular formula $C_{16}H_{16}O_6N_2S_3$, which has been tentatively assigned the structure IV. The nature of the sulphide link has not yet been established. 4-Chloro-3-ethoxy-nitrobenzene readily reacted with sodium hydrosulphide[11], though direct condensation yielded a mixture of the trisulphide $C_{16}H_{16}O_6N_2S_3$, described above, and 2:2′-diethoxy-4:4′-dinitrodiphenyl disulphide. A modified procedure, in which sodium 2-ethoxy-4-nitrothiophenoxide was first formed by the action of sodium hydrosulphide on 4-chloro-3-ethoxynitrobenzene and then condensed *in situ* with the latter substance, gave III in 30 per cent. yield (crude). Purification of the product obtained by this method was troublesome and wasteful, involving multiple chromatographic adsorptions and recrystallisations.

Compound III was more readily obtained in 21 per cent. over-all yield by an alternative route, the condensation of 2-acetylamino-4-chloro-5-ethoxynitrobenzene with sodium sulphide by the method of Hodgson and Dodson[10]; the product, a mixture of 5:5′-diacetylamino-2:2′-diethoxy-4:4′-dinitrodiphenyl sulphide and 5-acetylamino-5′-amino-2:2′-diethoxy-4:4′-dinitrodiphenyl sulphide was de-acetylated and de-aminated by the method of van Erp[12]. Condensation of sodium 5-acetylamino-2-ethoxy-4-nitrothiophenoxide with 2-acetylamino-4-chloro-5-ethoxynitrobenzene using the procedure described above, gave the same two monosulphides, although the higher yield anticipated by this method was not obtained.

The preliminary pharmacological tests which are recorded below were kindly carried out by Professor G. A. H. Buttle of this School.

In vitro tuberculostatic activities of 4:4′-diamino-2:2′-diethoxydiphenyl sulphone (I, R $=$ C_2H_5) and of 4:4′-diamino-2:2′-dihydroxydiphenyl sulphone (I, R$=$H, as dihydrochloride) were examined in Dubos medium using *M. tuberculosis* (H37Rv); sodium 4-aminosalicylate and 4:4′-diaminodiphenyl sulphone were used for comparison. The results are recorded in Table I.

A limited investigation has also been made of the *in vivo* activity of 4:4'-diamino-2:2'-dihydroxydiphenyl sulphone in mice infected intravenously with 0·01 mg. (dry bacterial substance) of *Mycobacterium murium* (NCTC 5676), with results which are summarised in Table II.

TABLE I

	Inhibitory Dilution
Substance I ($R=C_2H_5$)	1 : 16,000
Substance I (R = H)	1 : 256,000
4 : 4'-Diaminodiphenyl sulphone	1 : 128,000
Sodium 4-aminosalicylate	1 : 64,000

TABLE II

Substance	Number of Mice	Daily Dose mg.	Mortality after 3 weeks
4 : 4'-Diaminodiphenyl sulphone	6	1	3/6
Sodium 4-aminosalicylate...	6	1	3/6
4 : 4'-Diamino-2 : 2'-dihydroxydiphenyl sulphone	6	1	0/6*
Nil (controls)	6	0	4/6

* Post mortem examination of the survivors (killed after 3 weeks) revealed that four of the six mice were normal throughout.

Examination of the oral toxicity in mice of 4:4'-diamino-2:2'-dihydroxydiphenyl sulphone has shown it to be non-toxic at doses of 500 mg./kg., a dose level at which the parent compound, 4:4'-diaminodiphenyl sulphone is known to exhibit toxic reactions.

This work is part of a thesis presented to the University of London in May, 1950, for the degree of Ph.D.

EXPERIMENTAL

All m.pts. are uncorrected.

The following were prepared by the method of van Erp[12]:—

2-Acetylamino-4-chloro-5-ethoxynitrobenzene
4-Chloro-3-ethoxynitrobenzene
2-Chloro-5-nitrophenol.

4-Acetylamino-2-acetoxybenzenesulphinic Acid (II). 4-Acetylamino-2-acetoxybenzenesulphonyl chloride (16g., Thorpe and Williams[13]) was stirred with a solution of sodium sulphite ($Na_2SO_3,7H_2O$, 28·6 g.) in water (60 ml.). The solution was made just alkaline and maintained so for 2 hours at room temperature. The brown sludge which formed was removed by filtration and the filtrate mechanically stirred and acidified by the slow addition of 60 per cent. sulphuric acid. The crude product (7·34 g.), m.pt. 143°C. (decomp.) was precipitated as a buff crystalline solid, which on recrystallisation from ethyl alcohol (charcoal) gave white prisms, m.pt. 145°C. (decomp.) of *4-acetylamino-2-acetoxybenzene-*

sulphinic acid. Found: C. 45·5; H. 4·3; N, 5·7; S, 13·0 per cent. Eq.wt. 259. $C_{10}H_{11}O_5NS$ requires C. 46·7; H, 4·3; N, 5·5; S, 12·5 per cent. Eq.wt. 257.

Sodium salt. Found: Na,8·4 per cent. $C_{10}H_{10}O_5NSNa$ requires Na.8·2 per cent.

Condensation of 4-chloro-3-ethoxynitrobenzene with sodium sulphide. Sodium sulphide ($Na_2S,9H_2O$, 1·29 g.) dissolved in water (3 ml.) and ethyl alcohol (97 per cent. 2 ml.) was slowly added to a mechanically stirred solution of 4-chloro-3-ethoxynitrobenzene (2 g.) in alcohol (10 ml.). The solution. which immediately became deep red, commenced to deposit a yellow solid after being refluxed for 10 minutes. Refluxing was continued for another 2 hours. The crystalline precipitate (0·2 g.) was recrystallised from glacial acetic acid forming yellow platelets. m.pt. 209°C. Found: C, 45·3; H, 3·8; N, 6·6; S, 22·8 per cent. Mol.wt. (Rast) 492. $C_{16}H_{16}O_6N_2S_3$ requires C, 44·9; H, 3·8; N, 6·5; S, 22·5 per cent. Mol. wt. 428.

The filtrate was evaporated to dryness, the product dissolved in benzene and then chromatographed on alumina. The first, yellow benzene eluate gave, on concentration and crystallisation, yellow rosettes of 2-chloro-5-nitrophenol (0·95 g.) m.pt. and mixed m.pt. 121° to 122°C. Found: C, 41·7; H 1·8; N, 7·9; Cl, 19·7 per cent. Calc. for $C_6H_4O_3NCl$ C, 41·5; H, 2·3; N, 8·1; Cl, 20·4 per cent.

Condensation of 2-acetylamino-4-chloro-5-ethoxynitrobenzene with sodium sulphide. Sodium sulphide ($Na_2S,9H_2O$, 2·4 g.), dissolved in water (2·5 ml.) was slowly added to a stirred solution of 2-acetylamino-4-chloro-5-ethoxynitrobenzene in alcohol (97 per cent.) (20·5 ml.). After refluxing for one hour and cooling, the red solid (1·57 g.), m.pt. 210° to 218°C., which deposited, was separated into two components (A and B) by boiling with a limited volume (60 ml.) of 80 per cent. acetic acid.

A. On cooling, the acetic acid solution yielded red crystals (0·525 g.) m.pt. 180° to 195°C., which, after recrystallisation from 80 per cent. acetic acid formed orange crystals of *5-acetylamino-5′-amino-2:2′-diethoxy-4:4′-dinitrodiphenyl sulphide,* m.pt. 209°C. Found: C, 49·0; H, 4·7; N, 12·8 per cent. $C_{18}H_{20}O_7N_4S$ requires C. 49·5; H, 4·6; N, 12·8 per cent.

B. The insoluble fraction was recrystallised from a large volume of 80 per cent. acetic acid yielding yellowish-orange needles of *5 : 5′-diacetylamino-2 : 2′-diethoxy-4 : 4′-dinitrodiphenyl sulphide,* m.pt. 240° to 242·5°C. Found: C, 49·4; H, 4·2; N, 11·5; S, 6·5 per cent. $C_{20}H_{22}O8N_4S$ requires C. 50·2; H, 4·6; N, 11·7; S, 6·7 per cent.

5:5′-Diamino-2:2′-diethoxy-4:4′-dinitrodiphenyl Sulphide.

Method A. 2-Acetylamino-4-chloro-5-ethoxynitrobenzene (8·1 g.) was suspended in ethyl alcohol (97 per cent., 160 ml.) and refluxed for 8 hours with a solution of sodium sulphide ($Na_2S,9H_2O$, 3·8 g.) in water (8 ml.). The crude mixture of monosulphides, which separated after removal of the alcohol, was heated with 0·66 N alcoholic potassium hydroxide for

1 hour under reflux. On distillation of the alcohol, a solid product was obtained which formed red needles of *5:5′-diamino-2:2′-diethoxy-4:4′-dinitrodiphenyl sulphide* (2·98 g.), m.pt. 196° to 198°C., from 60 per cent. acetic acid. Found: C, 48·9; H, 4·7; N, 13·90; S, 8·0 per cent. $C_{16}H_{18}O_6N_2S$ requires C, 48·6; H, 4·6; N, 14·2; S, 8·1 per cent.

Method B. Sodium sulphide ($Na_2S,9H_2O$, 8·0 g.) dissolved in water (65 ml.) was neutralised by the careful addition of sodium bicarbonate (2·75 g.), the solution being cooled below 20°C. Sodium carbonate was precipitated by the slow addition of ice-cold methyl alcohol (65 ml.) and the filtrate (mainly sodium hydrosulphide) was refluxed with 2-acetyl-amino-4-chloro-5-ethoxynitrobenzene (8·36 g.) for 10 minutes, under nitrogen, to give a clear red solution. Sodium carbonate (1·73 g.) and 2-acetylamino-4-chloro-5-ethoxynitrobenzene (8·36 g.) were added and the solution stirred and heated under reflux for 2·5 hours. The crude product was treated as described under A above. Yield 5·68 g.

2 : 2′-Diethoxy-4 : 4′-dinitrodiphenyl disulphide. A solution of sodium hydrosulphide (3·06 g. $Na_2S.9H_2O$), prepared as described above, was refluxed for 4 hours with 4-chloro-3-ethoxynitrobenzene (2 g.). The solution was cooled and poured onto ice (100 g.) when a yellowish-green solid (0·37 g.) was precipitated, which, after recrystallisation from glacial acetic acid formed yellow platelets, m.pt. 209°C., identical with the trisulphide $C_{16}H_{16}O_6N_2S$ isolated previously. The filtrate was extracted with ether, the ether solution dried (anhydrous sodium sulphate) and the solvent removed. The yellow solid product was recrystallised from ethyl alcohol-acetone (2:1) forming bright yellow microcrystalline platelets (0·52 g.) m.pt. 152°C. of *2 : 2′-diethoxy-4 : 4′-dinitrodiphenyl disulphide*. Found: C, 48·5; H, 4·4; N, 6·9; S, 15·7 per cent. Mol.wt. (Rast) 348. $C_{16}H_{16}O_6N_2S_2$ requires C, 48·5; H, 4·1; N, 7·1; S, 16·2 per cent. Mol.wt. 394.

2 : 2′-Diethoxy-4 : 4′-dinitrodiphenyl Sulphide.

Method A. Nitrosylsulphuric acid (13 g.) prepared by the method of Gattermann and Liebermann[14] was slowly heated to 50°C. with 5:5′-diamino-2:2′-diethoxy-4:4′-dinitrodiphenyl sulphide (2 g.) in a 500 ml. flask and maintained at that temperature until the solid had completely dissolved (*ca.* 1/2 to 1 hour). The flask was cooled by immersion in ice and ice-cold ethyl alcohol (100 ml.) slowly added. After the effervescence had ceased, the flask was heated at 100°C. for 1/2 hour. Acetaldehyde and ethyl alcohol were removed by steam distillation and the black resinous mass which solidified on cooling was chromatographically adsorbed on alumina from acetone. Evaporation of the solvent from the first, reddish-yellow, acetone eluate yielded a brown solid, which, after recrystallisation from acetic acid (60 per cent.) (charcoal), formed yellow platelets (0·9 g.) of *2 : 2′-diethoxy-4 : 4′-dinitrodiphenyl sulphide,* m.pt. 115°C.

Method B. A solution of sodium hydrosulphide (≡14·2 g. $Na_2S.9H_2O$) was refluxed with 4-chloro-3-ethoxynitrobenzene (11·85 g.) for 5 minutes under nitrogen. Sodium carbonate (4·94 g.) and 4-chloro-3-ethoxynitro-

benzene (11·85 g.) was added and the solution stirred and heated under reflux for 16 hours. Methyl alcohol and unchanged 4-chloro-3-ethoxy-nitrobenzene (9·56 g.) were removed by steam distillation and the solid product extracted with boiling acetone. Evaporation of the acetone solution yielded a yellow solid, which, on recrystallisation from 60 per cent. acetic acid, gave crude 2:2′-diethoxy-4:4′-dinitrodiphenyl sulphide (7·28 g.) in yellow needles, m.pt. 108° to 115°C. A pure specimen for analysis was obtained by successive recrystallisation from 80 per cent. acetic acid (twice) and from benzene and light petroleum (b.pt. 60° to 80°C.) followed by chromatographic adsorption (twice) on alumina and elution in benzene, the first yellow eluate being collected. Evaporation of the benzene solution and recrystallisation of the product from 40 per cent. acetic acid yielded bright yellow needles of pure 2:2′-diethoxy-4:4′-dinitrodiphenyl sulphide, m.pt. 115°C. Found: C, 52·6; H, 4·5; N, 7·7; S, 8·1 per cent. $C_{16}H_{16}O_6N_2S$ requires C, 52·8; H, 4·4; N, 7·7; S, 8·8 per cent.

2 : 2′-Diethoxy-4 : 4′-dinitrodiphenyl Sulphone. Potassium permanganate (5·2 g.) in hot water (40 ml.) was added drop by drop to a stirred solution of 2:2′-diethoxy-4:4′-dinitrodiphenyl sulphide (8·18 g.) in boiling glacial acetic acid (120 ml.). Water (120 ml.) was added and the cooled mixture decolorised with sulphur dioxide. The pale yellow crystalline precipitate was redissolved in boiling glacial acetic acid (100 ml.) and reoxidised with potassium permanganate by the above procedure. Dilution and decolorisation of the cold solution yielded a white solid (4·06 g.), which, on recrystallisation from 60 per cent. acetic acid, formed shining white needles of *2 : 2′-diethoxy-4 : 4′-dinitrodiphenyl sulphone*, m.pt. 213°C. Found: C, 48·1; H, 4·2; N, 7·3; S, 8·3 per cent. $C_{16}H_{16}O_8N_2S$ requires C, 48·5; H, 4·0; N, 7·1; S, 8·1 per cent.

2 : 2′-Dihydroxy-4 : 4′-dinitrodiphenyl Sulphone. 2:2′-Diethoxy-4:4′-dinitrodiphenol sulphone (0·44 g.) was heated for 2 hours at 100°C. with concentrated sulphuric acid (1·2 g.). Upon cooling and pouring on to ice (20 g.), a white crystalline solid separated, which was recrystallised from 30 per cent. acetic acid (charcoal) to yield white needles of 2:2′-dihydroxy-4:4′-dinitrodiphenyl sulphone, m.pt. 233°C. Found: C, 42·0; H, 2·5; N, 8·2 per cent. $C_{12}H_8O_8N_2S$ requires C, 42·4; H. 2·4; N, 8·2 per cent.

4:4′-Diamino-2:2′-dihydroxydiphenyl Sulphone (I, R = H). 2:2′-Diethoxy-4:4′-dinitrodiphenyl sulphone (3·38 g.) was refluxed with constant-boiling hydriodic acid (100 ml.) at 140°C. for 24 hours, the solution being stirred continuously. The cooled solution was diluted with water (100 ml.), decolorised with sulphur dioxide and neutralised by the addition of sodium carbonate, when the product, a white solid (2·1 g.), was precipitated. The aqueous filtrate was extracted with ether, the ethereal solution washed with water, dried (anhydrous sodium sulphate) and evaporated to yield a further small quantity of product (0·1 g.). The combined products were crystallised from hot water forming small needles (1·7 g.), off-white in colour, of *4 : 4′-diamino-2 : 2′-dihydroxydiphenyl sulphone*, m.pt. 179° to 181°C. Found: C, 51·6; H, 4·4; N, 9·9; S, 11·1

per cent. $C_{12}H_{12}O_4N_2S$ requires C, 51·4; H, 4·3; N, 10·0; S, 11·4 per cent.

Hydrochloride $(C_{12}H_{12}O_4N_2S)$,2HCl found white prisms, m.pt. 207°C., from alcohol-ether. Found: C, 40·5; H, 4·5; N, 8·2 per cent. Eq. wt. 355. $C_{12}H_{14}O_4Cl_2N_2S$ requires C, 40·8; H, 4·0; N, 7·9 per cent. Eq. wt. 353.

4 : 4'-Diamino-2 : 2'-diethoxydiphenyl Sulphone (I, R = OC_2H_5). 2:2'-Diethoxy-4:4'-dinitrodiphenyl sulphone (3·38 g.) was refluxed with constant boiling hydriodic acid (100 ml.) for 7 hours at 140°C., the solution being stirred continuously. The cooled solution was diluted with water (100 ml.) and decolourised with sulphur dioxide. The cream-coloured precipitate of *4 : 4'-diamino-2 : 2'diethoxydiphenyl sulphone* was crystallised from 30 per cent. acetic acid, in small white needles, m.pt. 269°C. Found: C, 57·1; H, 5·9; N, 8·5 per cent. $C_{16}H_{20}O_4N_2S$ requires C, 57·1; H, 5·9; N, 8·3 per cent.

References

1. Smith, Jackson, Junge and Bhattacharya, *Amer. Rev. Tuberc.*, 1949, **60,** 62
2. Youmans and Doub, *ibid.*, 1946, **54,** 288.
3. Friedlander and French, *ibid.*, 1947, **56,** 360.
4. Berg, *J. chem. Soc.*, 1949, 1991.
5. Thorpe and Williams, *Biochem. J.*, 1941, **35,** 52.
6. Amstutz, *J. Amer. Chem. Soc.*, 1950, **72,** 3420.
7. Ferry, Buck and Baltzly, *Organic Syntheses,* Wiley, New York, 1942, **22,** 31.
8. Burton and Hu, *J. chem. Soc.*, 1948, 601.
9. Peak and Williams, *ibid.*, 1950, 445.
10. Hodgson and Dodson, *ibid.*, 1948, 1002.
11. Hodgson and Ward, *ibid.*, 1948, 242.
12. van Erp, *J. prakt. Chem.*, 1930, **127,** 34.
13. Thorpe and Williams, *Biochem. J.*, 1941, **35,** 61.
14. Gattermann and Liebermann, *Liebigs Ann.*, 1912, **193,** 200.

THE CHEMISTRY OF ANTI-PERNICIOUS ANÆMIA FACTORS

PART VI. THE MODE OF COMBINATION OF *COMPONENT* α IN VITAMIN B_{12}

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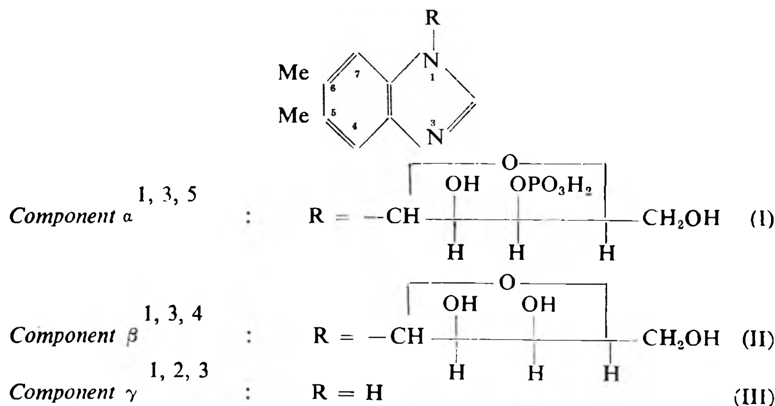
IN the course of studies on the acidolysis of vitamin B_{12}, Beaven *et al.* (Part III[1]) established the formation of three closely related hydrolytic products which they termed *components* α, β and γ. Comparison of the absorption spectra of these compounds with those of known heterocycles led to the identification of *component* γ with 5:6-dimethylbenziminazole (III), a conclusion also reached by Brink and Folkers[2], and of *components* α and β as 1-substituted 5:6-dimethylbenziminazoles. In addition, the view was expressed that the two latter compounds might well prove to be sugar derivatives of 5:6-dimethylbenziminazole.

Subsequent work outlined in Part V[3], and the independent studies of Brink, Holly, Shunk, Peel, Cahill and Folkers[4], and of Buchanan, Johnson, Mills and Todd[5], have now established the main structural features of the *component* α and β molecules. Thus the constitution of a 5:6-dimethylbenziminazole-1-α_a-D-ribofuranoside (II)* has been assigned to the latter compound[4,5] and the formulation confirmed by direct synthesis[4]. The structure of *component* α, however, is based on less secure experimental evidence. The compound is undoubtedly the 2′ or 3′ phosphoryl derivative of (II)[3,5], the 3′-formulation (I) being, in our view, the preferred structure.

Acidolysis of B_{12} thus leads to initial formation[1,3] of *component* α, which then undergoes stepwise hydrolysis into *component* β, and finally into *component* γ.

Structure of Components α, β *and* γ



*See footnote on page 954

By using the extinction coefficient of 5:6-dimethylbenziminazole (III) as a model for reckoning molar extinctions, Beaven *et al.*[1] concluded that one molecule of vitamin B_{12} gives rise to approximately one molecule of 5:6-dimethylbenziminazole (calc. as *components* α, β and γ) on acidolysis. In addition, the recognition of two bands at λ = 2885Å and 2785Å in the B_{12} absorption spectrum as characteristic of the benziminazole chromophore led them to the further postulate that a benziminazole nucleus exists preformed in the B_{12} molecule.

We now find that the synthetic 5:6-dimethylbenziminazole glycosides (IV) described in Parts IV[6] and V[3] of this series possess absorption spectra indistinguishable from each other and from those of *components* α and β. The absorption spectrum of such a 5:6-dimethylbenziminazole glycoside, together with the curve for vitamin B_{12}, is shown in Figure 1. It is now clear that the absorptivity of B_{12} in the region of absorption of the benziminazole is not sufficiently high to accommodate two equivalents of the latter. We may thus conclude, with confidence, that vitamin B_{12} contains but one preformed benziminazole nucleus in its molecule. This nucleus, in our view, consists of the intact *component* α, structure (I), as only by this assumption does it appear possible to explain the facility with which this fragment is released on acidolysis[3].

The Anomaly Present in the B_{12} Absorption Spectrum

Inspection of the curves shown in Figure 1 reveals an anomaly. Thus whereas the benziminazole curve (2) is characterised by a well-marked

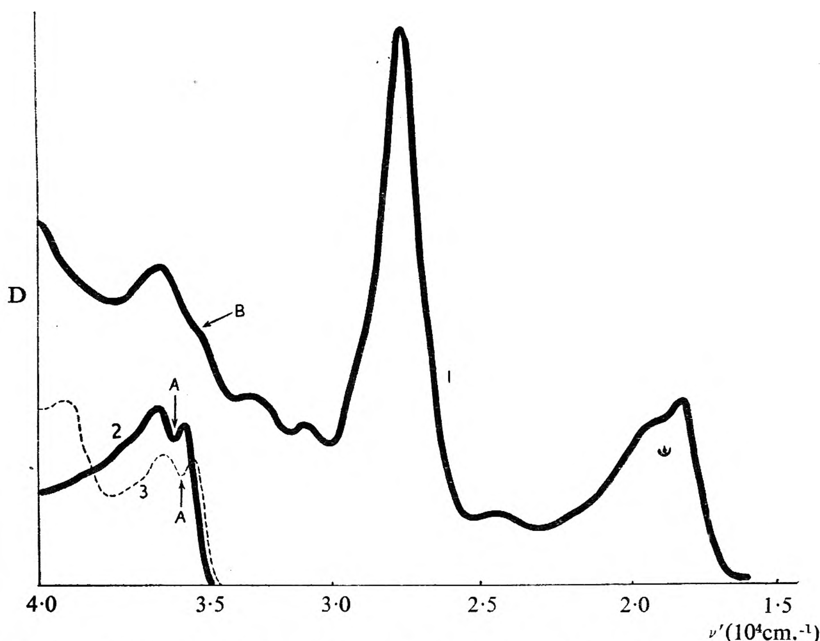


FIG. 1. Absorption spectra of 1. Vitamin B_{12}, 2. 5:6-dimethylbenziminazole-1-glycoside (*p*H 2), 3. 5:6-dimethylbenziminazole-1-glycoside (*p*H 12).

narrow band and "notch" (marked A), the curve for vitamin B_{12} shows only an inflection (marked B) at $\lambda = 2885$Å. Since the absorption of a mixture of two chromophore-bearing compounds is additive provided there is no interaction between them, the non-appearance of the "notch" in the absorption curve of vitamin B_{12} must be interpreted in one of two ways: (i) the absorption spectrum of the rest of the B_{12} molecule, which we shall term the "coloured fragment," masks the benziminazole band; or (ii) the contribution of the benziminazole chromophore to the B_{12} spectrum is altered by some combination or linkage of *component* α within the B_{12} molecule.

The first interpretation is unlikely. It can be shown graphically that for this situation to obtain the absorption curve of the "coloured fragment" must possess a small band which "fills up"—for want of a better term—the small minimum (A; curve 2) present in the benziminazole spectrum. Interpretation (ii), however, requires closer study.

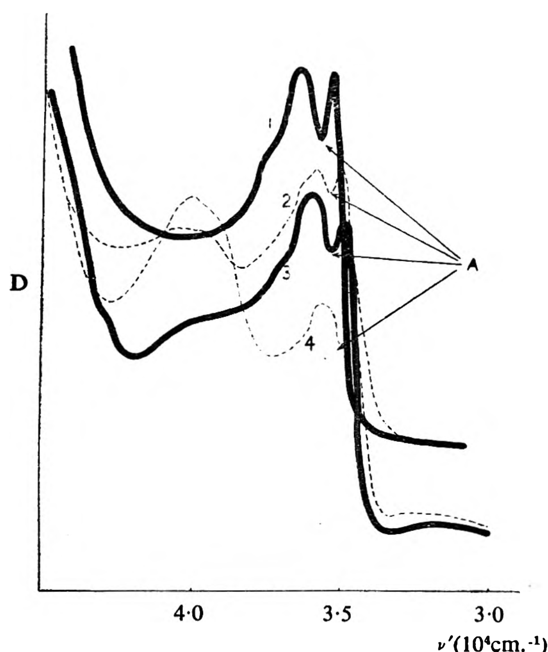
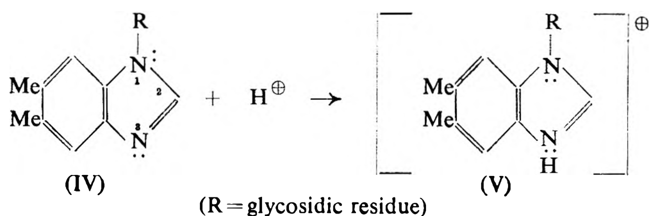


FIG. 2. Absorption curves of 1. *Component* γ (*p*H 2), 2. *Component* γ (*p*H 12), 3. *Components* α and β (*p*H 2), 4. *Components* α and β (*p*H 12).

We already know[1] that the "notch" in question (marked A) is resolved in the spectra of *components* α, β and γ (Figure 2). In addition, it is quite certain that the resolution of the "notch" in the *component* α spectrum will not be impaired by possible linkage of the phosphoryl residue to the rest of the B_{12} molecule (*vide infra*). Consideration of the ways in which the chromophoric character of *component* α can be affected sufficiently to abolish the resolution of the "notch" without interfering with the wavelength position of the main band and its subsidiary features leads, by a process of elimination, to the conclusion that the nitrogen atom in position 3 of the benziminazole nucleus in *component* α (I), is involved in some form of combination. Evidence supporting this view is recorded in the following section.

Observations Regarding the Dissociation Constants of Vitamin B_{12} and of Certain Benziminazoles

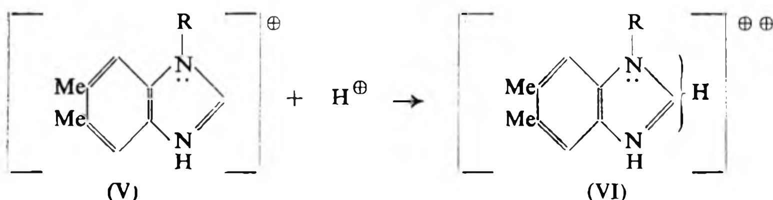
The benziminazole nucleus contains two basic nitrogen atoms and can thus, theoretically, accept a maximum of two protons. Detailed electrometric studies by Davies, Mamalis, Petrow and Sturgeon[7] show that benziminazole, its alkyl derivatives, and its alkylated-1-glycosides, have pK_a values in the region of 5. This pK_a, subsequently referred to as pK_{a_1}, corresponds to the



(R = glycosidic residue)

acceptance of one proton by the benziminazole (IV) to give the benziminazolinium ion (V) ($pK_{a_1} \simeq 5{\cdot}0$). The electron pair available on the nitrogen atom at position 3, subsequently referred to as the N^3 nitrogen, is utilised for this purpose, addition of proton being accompanied by a marked shift in the position of the absorption bands as shown in Figure I, curves 2 and 3.

By spectrometric titration we now find that the foregoing benziminazole derivatives (IV) give evidence for a second dissociation constant in the region of pH 0·0. Thus when the concentration of hydrochloric acid is raised to normal, a new spectral change occurs which consists of a shift to longer wavelengths. The effect is still more marked in 5 N and 10 N hydrochloric acids.

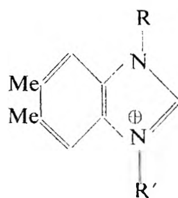


This second pK_a, subsequently referred to as pK_{a_2}, is clearly dependent upon the benziminazolinium ion (V) accepting a proton to give (VI), which will obviously exist only in a very high hydrogen ion concentration.

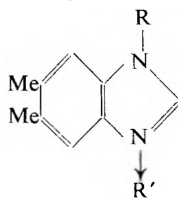
Bearing these facts in mind, examination of the absorption spectra in Figure 1 leads to the following conclusions: (i) The synthetic 5:6-dimethylbenziminazole-1-glycoside (IV) shows, as expected, a pronounced wavelength shift in passing from pH 12 (curve 3) to pH 2 (curve 2). This spectral shift corresponds to the existence of a pK_{a_1} of *ca.* 5·0 (i.e., IV→V). (ii) The inflection at $\lambda = 2885$Å in the vitamin B_{12} spectrum, for which the benziminazole chromophore present in (I) is responsible, fails to show a wavelength shift in passing from pH 12 to pH 2. On increasing the acid concentration to 0·1 N, the B_{12} spectrum undergoes a

definite but reversible shift to shorter wavelengths. Further increase in acid concentration leads to spectral changes which are partly irreversible.

Consideration of these facts leads to the conclusion that the "*component* α combination" existing in the B_{12} molecule differs from the 5:6-dimethylbenziminazole-1-glycoside (IV) by the absence of a pK_{a_1}, as defined on p. 947, but resembles (V) by giving the evidence of a pK_{a2} at a pH of *ca.* 0·0. This fact must surely mean that N^3 in the "*component* α combination" must lack the free electron pair responsible for the pK_{a_1}. If this is indeed the case, the conclusion that the N^3 electron pair is involved in some form of linkage within the B_{12} molecule appears to be inescapable. Theoretically, two types of linkage are indicated:



(VII)



(VIII)

(i) the N^3 electron pair may be shared with a grouping R′ in the form of a covalent bond giving a compound of the quaternary salt type (VII), or (ii) the N^3 electron pair may be donated to a cationic grouping R′ in the form of a coordinate link as indicated in (VIII).

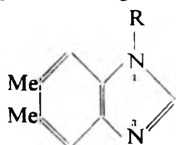
Before discussing these alternative formulations, it is perhaps pertinent to restate once more the spectroscopic features which distinguish the "*component* α combination" existing in B_{12} from those of a simple 5:6-dimethylbenziminazole-1-glycoside (IV). Briefly, these differences involve: (i) The absence of a spectral shift in passing from pH 12 to pH 2 (Figure 1, compare curve 1 with curves 2 and 3), i.e., the absence of a pK_{a_1} and (ii) The absence of the "notch" (Figure I; marked A in curves 2 and 3) characteristic of (IV) but evident in B_{12} as an inflection (Figure 1; marked B).

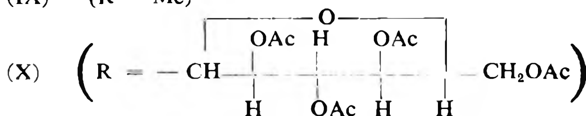
Returning now to a consideration of (VII) and (VIII), substances possessing either structure may confidently be expected to lack a pK_{a_1} and thus satisfy the first of the conditions formulated above. The effect of the N^3-R′ linkages on the resolution of the benziminazole "notch," however, cannot be predicted at this stage, but must be determined experimentally by spectroscopic study of model compounds.

THE EFFECT OF QUATERNATION ON THE ABSORPTION SPECTRA OF 1-SUBSTITUTED 5:6-DIMETHYLBENZIMINAZOLES

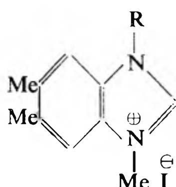
The absorption spectra of: 1:5:6-trimethylbenziminazole (IX) and of 5:6-dimethylbenziminazole-1-β-tetraacetyl-D-glucopyranoside[6] (X) are given in Figure 3. Both compounds possess pK_{a_1} dissociation constants

(IX) (R = Me)

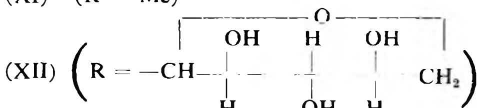




and thus show pronounced spectral shifts in passing from pH 12 (curves 2 and 4, respectively) to pH 2 (curves 1 and 3, respectively). In addition, both compounds show a well-resolved " notch " (marked A) in the region of λ = 2885Å. The " notch," it will be noted, is more pronounced



(XI) (R = Me)



in the case of (IX) (curves 1 and 2), a difference no doubt due to the contrasting inductive effects of the 1-methyl (+I) and 1-glycosido (−I) substituents on the benziminazole chromophore.

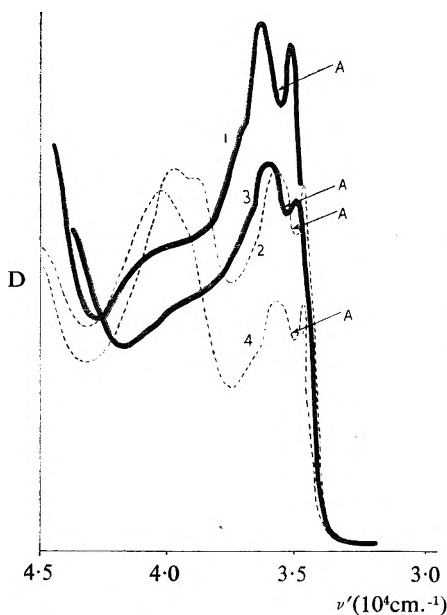


FIG. 3. Absorption curves of 1. 1:5:6-trimethylbenziminazole (pH 2), 2. 1:5:6-trimethylbenziminazole (pH 12), 3. 5:6-dimethylbenziminazole - 1 - β - tetra-acetyl-D-glucopyranoside (pH 2), 4. 5:6-dimethylbenziminazole - 1 - β - tetra-acetyl-D-glucopyranoside (pH 12).

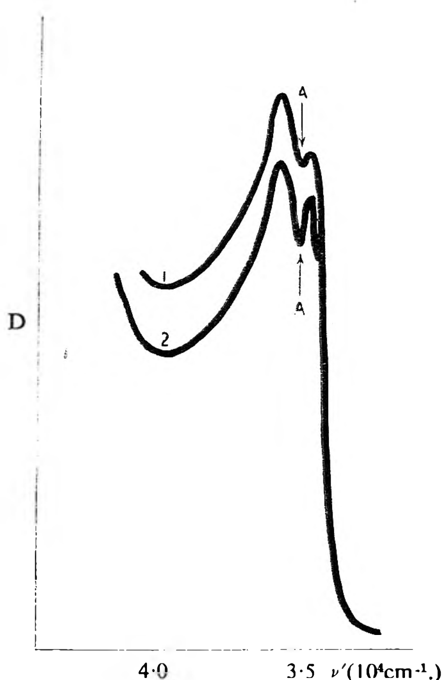


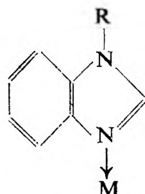
FIG. 4. 1. 5:6-dimethylbenziminazole-1-β-D-arabopyranoside-3-methiodide (pH 2 and 12), 2. 1:5:6-trimethylbenziminazole-3-methiodide (pH 2 and 12).

Quaternation of the N^3 atom leads, as expected, to the abolition of the pK_{a_1}. This point is evident from the absorption spectra of 1:5:6-trimethylbenziminazole 3-methiodide (X) (Figure 4; curve 2) and of 5:6-dimethylbenziminazole-1-β-D-arabopyranoside 3-methiodide (XII) (Figure 4; curve 1) which, respectively, give identical absorption spectra in both alkaline and acid solution. Quaternation fails, however, to abolish the well-resolved " notch " (marked A) which is clearly

present in the absorption spectra of both these compounds. The spectroscopic features of 1-substituted 5:6-dimethylbenziminazole-3-quaternary salts are thus incompatible with the second of the conditions formulated on p. 948. The stability of such compounds as (XI) and (XII) towards hydrochloric acid, too, stands in marked contrast to the facility with which release of *component* α occurs under the influence of acid. 1-Substituted 5:6-dimethylbenziminazole-3-quaternary salts may therefore be excluded from further consideration.

THE EFFECT OF COORDINATION BY THE N^3 ATOM ON THE ABSORPTION SPECTRA OF 1-SUBSTITUTED BENZIMINAZOLES

Considerable difficulty was experienced in preparing model compounds of type (XIII)



(M = Metal) (XIII)

for spectroscopic study. Although complex formation between the base and such compounds as cobalt chloride was achieved in many cases[6], the resulting products proved, in general, to be compounds of low stability which dissociated into their component parts during solution prior to spectroscopic study. Discussion of these results is accordingly postponed. Complexes formed employing platinic chloride[8], however, proved sufficiently stable for our purpose and, moreover, contained the structural feature (VIII).

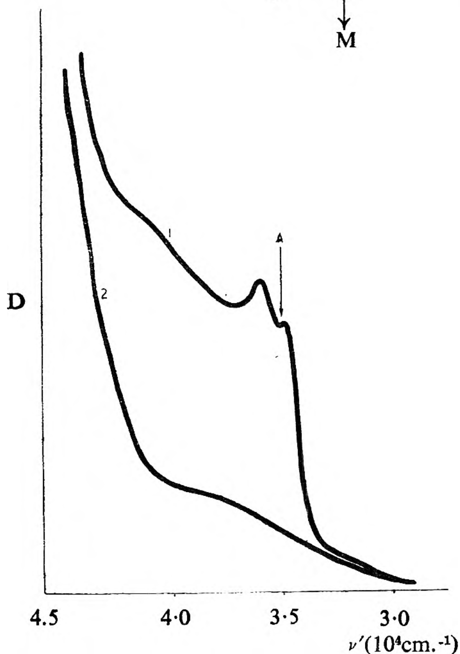


FIG. 5. 1. Platinum complex of 5:6-dimethylbenziminazole - 1 - arabopyranoside, 2. [Pt $(NH_2)_2$ Cl_4]. Curve 2 is given to indicate the absorption characteristics of a platinum complex which does not contain another specific chromophore.

The absorption spectrum of the 5:6-dimethylbenziminazole-1-α-L-arabopyranoside/platinic chloride complex is shown in Figure 5. Examination of the curve reveals: (i) the absence of an acid/alkali spectral shift, i.e., the absence of a pK_{a1}, as defined on p. 947 and (ii) a marked decrease in resolution of the long wave fine structure maximum at $\lambda = 2860$ Å (marked A).

The spectroscopic characteristics of compounds of type (XIII) (and hence VIII) thus fully satisfy the two conditions formulated on p. 948. We may therefore conclude, with reasonable certainty, that the N^3 electron pair in *component* α is deployed in some form of co-ordinate link in the combination existing in B_{12}. Evidence regarding the nature of this linkage is supplied by studies on the action of cyanide on vitamin B_{12}.

THE EFFECT OF CYANIDE ON VITAMIN B_{12}

When potassium cyanide or hydrocyanic acid is added in excess to a solution of vitamin B_{12}, the colour changes from red to deep violet with a correspondingly marked change in the absorption spectrum both in the visible and ultraviolet regions (*vide infra.*). The B_{12} cyanide complex so formed is stable only in solution in the presence of excess of cyanide, reverting to a red compound on reducing the *p*H to below 7, or, when hydrogen cyanide is employed, on simply allowing the solution to evaporate. The identity with vitamin B_{12} of the material so recovered is established by its absorption spectrum, its general crystallographic appearance, its behaviour on paper chromatograms[9], and its microbiological activity as measured with *Lactobacillus lactis* Dorner A.T.C.C. 10697. For this latter determination, we are indebted to Dr. S. W. F. Underhill and Miss F. E. Larkin. We may therefore conclude that: (i) cyanide adds reversibly to the B_{12} molecule, (ii) addition of cyanide occurs at an electrophilic centre in the B_{12} molecule, and (iii) addition is not accompanied by profound structural changes in the molecule.

Evidence concerning the point of attachment of cyanide is furnished by the observation that the coloured cobalt-containing fragment obtained by Ellis, Petrow and Snook[10] by acidolysis of B_{12} likewise adds cyanide under similar experimental conditions. The cobalt-containing part of the B_{12} molecule must therefore be the seat of the electrophilic centre involved in "B_{12}-cyanide complex" formation. This centre is, in our view, the central cobalt atom, the cyanophilic properties of which are well established and require no further comment (cf. Ephraim, *Inorganic Chemistry*, 3rd English Edition, 1939, p. 302).

The co-ordination of cyanide ion with the central cobalt atom, as shown diagrammatically in formula (XIV),

$$\text{—Co—} + \ddot{\text{C}}\text{N} \rightleftharpoons \underset{\underset{\text{CN}}{\uparrow}}{\text{—Co—}}$$

(XIV.)

is accompanied by profound changes in the B_{12} absorption spectrum as shown in Figure 6 (*vide supra*). Comparison of curves 1 and 2 (Figure 6) reveals the important fact that the benziminazole inflection (marked B) at $\lambda = 2885$Å present in the B_{12} curve (curve 1) has been replaced in the "B_{12}-cyanide complex" by a well-resolved notch (marked A) (curve 2). In fact, the *component* α contribution to the absorption spectrum of the "B_{12}-cyanide complex" may be considered as "normal," and the corresponding anomaly previously observed in the B_{12} spectrum (cf.

Figure 1) as absent. The co-ordination of cyanide ion with cobalt is thus able to influence directly the contribution made by the benziminazole chromophore to the B_{12} spectrum. Some form of linkage between the cobalt atom or chromophore and the benziminazole chromophore is thus established.

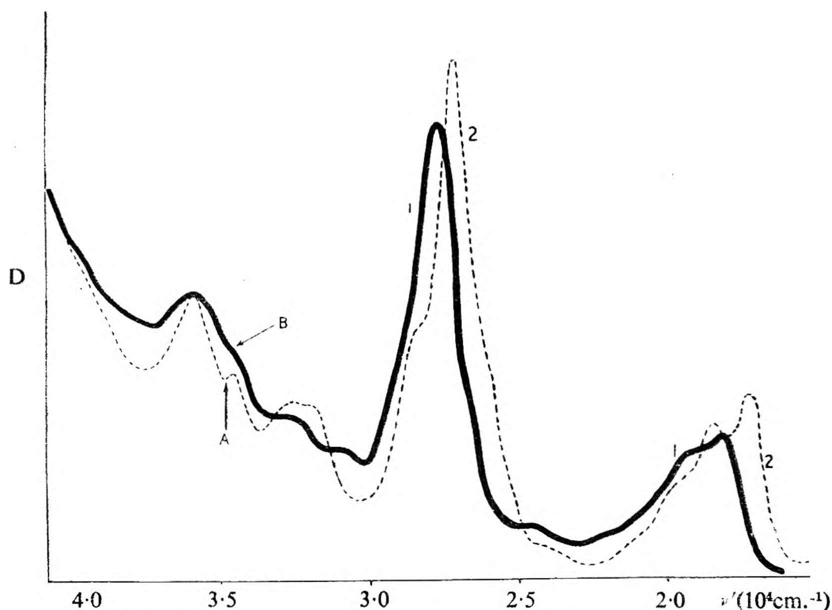


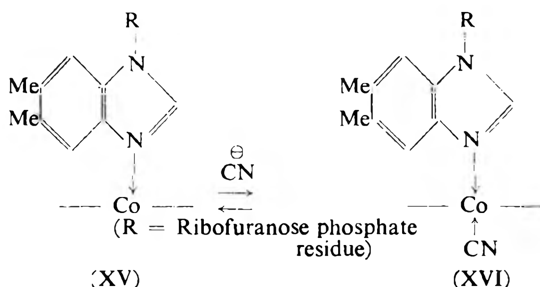
FIG. 6. 1. Vitamin B_{12}, 2. Vitamin B_{12}-cyanide complex.

The benziminazole chromophore (I), for reasons outlined above, is considered as bound through its N^3 atom by means of a co-ordinate linkage as shown in (VIII). The N^3 atom of the latter compound must therefore be involved in co-ordinate linkage with either the cobalt atom or chromophore. An unequivocal decision between these two alternative sites of combination is, unfortunately, impossible at the present time, owing to an almost complete absence of information regarding the detailed structure of the cobalt chromophore. The probable existence of a structural analogy between B_{12} and the hæmatin group of compounds, however, to which Ellis, Petrow and Snook[10] have already drawn attention, points to the hypothesis that N^3 in the benziminazole glycoside (I) is co-ordinately linked to cobalt as shown in (XV).

It is perhaps relevant to add that we visualise the cobalt-containing fragment as a planar structure somewhat akin spatially to a porphyrin, with *component* α lying perpendicularly to this plane.

The resolution of the benziminazole " notch " (Figure 6) in the " B_{12}-cyanide complex " spectrum is readily explained on this basis by assuming that combination of cyanide ion with cobalt to give (XVI) leads to an increase in the electronegativity of the cobalt atom and a corresponding decrease in the electronic contribution of the N^3 benziminazole nitrogen

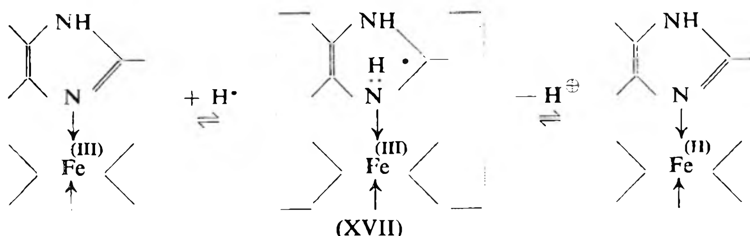
to the cobalt electron cloud. Decrease of this electronic contribution will, conversely, diminish the changes produced by the N^3 - Co linkage in the benziminazole chromophore spectrum and lead to an absorption curve more closely resembling that of an unattached 5:6-dimethylbenziminazole-1-glycoside (IV), as is indeed found to be the case. Thus the co-ordination hypothesis fulfils all the spectroscopic desiderata established during the course of this investigation. Its unequivocal proof, however, must await a closer knowledge of the internal architecture of the B_{12} molecule.



A precedent for a similar co-ordination is furnished by hæmoglobin and by cytochrome c in which a histidine residue is co-ordinated with the porphyrin iron atom. The analogy with hæm compounds is further strengthened by the recent observation of Wallman, Cunningham and Calvin[11], and of Gruen and Menassé[12], who have shown that the cobalt atom in vitamin B_{12} is trivalent and possesses octahedral symmetry. The effect of cyanide on the spectrum of B_{12} is likewise reminiscent of the effect of cyanide on hæm compounds described by J. Keilin[13], and of cyanide on cobalt porphyrins described by Taylor[14].

Some Implications of the Coordination Hypothesis

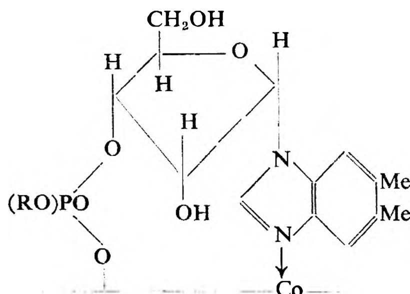
The function of the histidine residue in cytochrome c is to provide a channel for the transmission of electrons to and from the iron atom as indicated in the following scheme[15]:



whereby reversible reduction of the ferric atom is achieved. We are tempted to suggest a similar function to the benziminazole residue in vitamin B_{12} in view of the close and unexpected analogy evident between the two partial structures (XV) and (XVII). Cobalt co-ordination complexes, it is true, exist normally in the more stable cobaltic form. Nevertheless, the possibility that transient reduction of cobaltic to cobaltous

occurs in vitamin B_{12} by electron transmission through the benziminazole nucleus should not be excluded from consideration. It is of interest to note, in this connection, that the oxidation/reduction potential of the $Co^{++} \rightarrow Co^{+++}$ reaction is very much altered by conversion of the simple inorganic cobalt ion into a complex of the covalent octahedral type (see Pauling, *Nature of the Chemical Bond*, 2nd Ed., 1942, p. 96).

A point of difference between the behaviour of the histidine residue in the hæm pigments and the benziminazole residue in vitamin B_{12} is furnished by the action of dilute acids. Hydrolysis occurs in both cases. In B_{12}, however, progressive release of *component* α seems to take place in contrast to an instantaneous cleavage effect observed with hæm compounds. This difference in behaviour would appear to indicate the presence of an additional less readily hydrolysed linkage which, together with the N^3-Co link (cf. XV), binds *component* α to the rest of the B_{12} molecule. Buchanan, Johnson, Mills and Todd[5] have indeed proposed that the phosphoryl grouping in *component* α is joined directly to the cobalt macrofragment and also to the " ninhydrin-reacting fragment "[16] (D-1-amino-2-propanol[17, 18]) in tertiary union. Combination with D-1-amino-2-propanol in this fashion is, of course, inconsistent with evidence submitted by Cooley *et al.* in Part V[3]. Nevertheless, we accept the suggestion regarding the attachment of the phosphoryl residue to the cobalt-containing fragment, either directly or through an intermediate molecule, such as D-1-amino-2-propanol, and tentatively propose a two-fold attachment of *component* α as indicated below (XVIII).



(XVIII)

The feasibility of this formulation depends not only upon structural features of which we have no present knowledge, but also upon the stereochemical configuration of the glycosidic centre present in *component* α. Two-fold attachment can only be postulated for one of the anomeric forms of (I). This anomer* must possess the α_a glycosidic configuration established by Butler, Smith and Stacey[19] for the α_a and β_a forms of tetraacetyl-D-galacto-pyranose anilide. The anomeric form of

* In order to avoid ambiguity between the use of α, β γ to designate the hydroyltic products of B_{12}, and the use of α and β to designate the anomeric forms of the benziminazole glycosides, the latter have been termed the α_a and β_a-glycosides. The subscript a implies reference to the anomeric forms of sugars as generally accepted in carbohydrate nomenclature.

component α is not, of course, known with certainty. Nevertheless, it is perhaps more than a coincidence that Brink *et al.*[4] have provisionally assigned it the α_a-configuration on grounds of general usage and practice.

Summary and Conclusions

1. Spectroscopic studies lead to the conclusion that the nitrogen atom in position 3 of *component* α (I) is involved in a linkage of co-ordinate type within the B_{12} molecule.

2. Cyanide ion is shown to add reversibly to the cobalt-containing chromophore present in vitamin B_{12}, such addition being accompanied by a significant change in the anomalous contribution of the benziminazole chromophore of (I) to the ultraviolet absorption spectrum of vitamin B_{12}.

3. It is suggested that the nitrogen atom in position 3 of *component* α (I) is co-ordinately linked to cobalt as shown in (XVIII).

The authors thank Dr. B. Sturgeon and Mr. P. Mamalis for the preparation of the model compounds required for this investigation, and the Directors of The British Drug Houses Ltd., for encouraging this work.

NOTE. After Part VI had been submitted for publication, Brink, Kuehl and Folkers (*Science*, 1950, **112**, 354) presented evidence which indicates that a cyano-grouping is bound directly to the central cobalt atom in vitamin B_{12}. This fact in no way invalidates the arguments outlined in Part VI. The purple complex formed by addition of cyanide to vitamin B_{12}, however, which we refer to as the " B_{12}-cyanide complex," will therefore contain two cyano-groups linked directly to cobalt, one of which is readily lost with regeneration of the parent vitamin.

References

1. Beaven, Holiday, Johnson, Ellis, Mamalis, Petrow and Sturgeon, *J. Pharm. Pharmacol.*, 1949, **1**, 957.
2. Brink and Folkers, *J. Amer. chem. Soc.*, 1949, **71**, 2951.
3. Cooley, Ellis, Mamalis, Petrow and Sturgeon, *J. Pharm. Pharmacol.*, 1950, **2**, 579.
4. Brink, Holly, Shunk, Peel, Cahill and Folkers, *J. Amer. chem. Soc*. 1950, **72**, 1866.
5. Buchanan, Johnson, Mills and Todd, *Chem. and Ind.*, 1950, **22**, 426.
6. Mamalis, Petrow and Sturgeon, *J. Pharm. Pharmacol.*, 1950, **2**, 491, 503, 512.
7. Davies, Mamalis, Petrow and Sturgeon, *forthcoming publication.*
8. Davies *et al.*, *unpublished work.*
9. Woodruff and Foster, *J. biol. Chem.*, 1950, **183**, 569.
10. Ellis, Petrow and Snook, *J. Pharm. Pharmacol.*, 1949, **1**, 60.
11. Wallman, Cunningham and Calvin, *Private communication.*
12. Cruen and Menassé, *Experientia*, 1950, **6**, 263.
13. J. Keilin, *Biochem. J.*, 1949, **45**, 440; 1950, **6**, 263.
14. Taylor, *J. biol. Chem.*, 1940, **135**, 569.
15. After Thorell, *Ark. Kemis, Min., Geol.*, 1942, **16A**, (14).
16. Ellis, Petrow and Snook, *J. Pharm. Pharmacol.*, 1949, **1**, 950.
17. Wolf, Jones, Valiant and Folkers, *J. Amer. chem. Soc.*, 1950, **72**, 2820.
18. Cooley, Ellis and Petrow, *J. Pharm. Pharmacol.*, 1950, **2**, 535.
19. Butler, Smith and Stacey, *J. chem. Soc.*, 1949, 3371, c.f. Furberg, *Acta Crystallographica*, 1950, **3**, 325.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Acetophenone and Lobeline, Determination of. K. Matérn and G. Schill. (*Svensk farm. Tidskr.*, 1950, **54**, 445, 462.) Acetophenone is determined photometrically through the dinitrophenylhydrazone. Details are as follows. 5 ml. of a solution containing 0·015 to 0·040 mg. of acetophenone is treated with 5 ml. of 0·1 per cent. solution of 2-4-dinitrophenylhydrazine in 2N hydrochloric acid, and allowed to stand for 2 hours; 10 ml. of carbon tetrachloride is added, and the mixture is shaken strongly for 10 minutes. After removal of the acid layer, the tetrachloride solution is dried by shaking strongly with 50 ml. of water. Washing is repeated 3 times, each time with 50 ml. of water. The carbon tetrachloride solution is washed with sodium sulphate and the extinction is determined at 370 mμ. Correction is made for a blank test carried out in a similar manner. The method cannot be used for quantities of acetophenone less than 0·015 mg., as the reaction with dinitrophenylhydrazine is then too slow. For the determination of lobeline an aqueous solution, containing 1 to 2·5 mg. of lobeline hydrochloride, is treated with 20 ml. of buffer solution (*p*H,8·0) and made up to 70 ml. with water. The flask containing the mixture is then connected to a condenser with a receiver containing 10 ml. of water. After heating on a water-bath for 15 minutes, the solution is distilled over, at a rate of not more than 3 ml. per minute, until 30 ml. of distillate has been collected. The condenser is rinsed with a little water, the solution is made up to 100 ml., and 5 ml. of this solution is treated as above. For determining unchanged lobeline in partially decomposed solutions it is necessary first to remove any free acetophenone by shaking the slightly acidified solution twice with carbon tetrachloride. Direct determination of acetophenone in such solutions is unsatisfactory. The presence of alcohol interferes with the determination, giving results which are too high. G. M.

***p*-Aminosalicylic Acid, Identification of.** F. v. Bruchhausen, H. Karbe and W. Kunz. (*Arch. Pharm., Berl.*, 1950, **283**, 110.) The *p*-aminosalicylic acid used therapeutically is 4-amino-2-hydroxybenzoic acid. Some samples on the market have been found to be 5-amino-2-hydroxybenzoic acid. The following reactions may be used to identify the latter acid. Viebock reaction: by boiling with acid, the 5-aminosalicylic acid is converted into *p*-aminophenol; the solution is treated with hypochlorite and then with phenol; a blue colour is produced. Vlezenbeek reaction: to 0·01 g. of the compound is added 0·1 g. of resorcin and 1 ml. of concentrated sulphuric acid, and the mixture is heated to 180°C. After cooling, the mixture is poured into 5 ml. of water, made alkaline, cooled and a few drops of iodine solution added; a blue-violet colour appears, owing to formation of resorufin and resazurin. Reduction test: 5-aminosalicylic acid reduces ammoniacal silver solution; the true *para* compound does not. G. M.

Antrycide, Determination of. A. Spinks. (*Biochem. J.*, 1950, **47,** 299.) Four methods for the determination of antrycide are described. Two are colorimetric methods which may only be used for aqueous solutions of the drug. The antrycide solution (up to 10 µg. of antrycide/ml.) is well shaken with a solution of congo red WS, phosphate buffer (*p*H7) and butyl alcohol. After separation the upper layer is transferred to a colorimeter and the extinction coefficient is measured against pure butyl alcohol using a green filter. In the second method the antrycide solution is well mixed with a solution of suramin and phosphate buffer and set aside for 5 minutes. A solution of 2-*p*-dimethylaminosteyryl-6-acetamidoquinoline methochloride, SQ 24 (12·5 mg./100 ml.) is then added and the extinction coefficient is measured against distilled water at 505 mµ. The third method utilised the fluorescence of solutions of antrycide. The plasma to be analysed is compared with ordinary plasma and plasma containing a known amount of antrycide. The solutions are treated with trichloracetic acid, set aside for 10 minutes, centrifuged and the upper layer examined in a fluorimeter. For amounts varying from 1 to 7 µg. of antrycide the recovery was from 89 to **100** to 119 per cent. In the fourth method the plasma is diluted with water, treated with trichloracetic acid and centrifuged, and the upper layer is mixed with buffered eosin reagent and solution of *n*-butyl alcohol in chloroform. After vigorous shaking the lower layer is pipetted off, filtered and examined in a Coleman fluorimeter. Concentrations of 40 µg. of antrycide/1. may be determined with satisfactory accuracy. The method is fairly specific, most other tertiary and quaternary bases not reacting. It can also be applied to tissues and urine. A. D. O.

Carbon Monoxide, Spectrophotometric Determination of. N. C. Klendshoj, M. Feldstein and A. L. Sprague. (*J. biol. Chem.*, 1950, **183,** 297.) This method depends on the different value of the ratio between the optical densities of reduced hæmoglobin (3·15) at 555 and 480 mµ and that for carboxyhæmoglobin (1·94). Mixtures of the two pigments give intermediate values. 1 ml. of oxalated blood was diluted to 100 ml. with 0·4 per cent. ammonia and 3 ml. was placed in a cuvette with 10 mg. of sodium hydrosulphite. The solution was gently mixed and the densities at the two wavelengths were measured using 0·4 per cent. ammonia as a blank on a Beckman DU quartz spectrophotometer in 1 cm quartz cells. The ratio D_{555}/D_{480} was calculated from a calibration curve prepared from samples of blood containing known amounts of carbon monoxide. All determinations were checked by the Slyke's gasometric method and good agreement was obtained, especially when the content of carbon monoxide was less than 20 per cent. Methæmoglobin did not interfere with the results, but the method was not practicable when samples had become hæmolysed.

A. D. O.

Cocaine, Colour Test for. E. Rathenasinkam. (*Analyst*, 1950, **75,** 169.) The colour reaction with alkalis of the nitro-compound produced by nitration of cocaine with a mixture of nitric and sulphuric acids is used as a basis of a colour test. To about 0·5 mg. of the substance about 100 mg. of potassium nitrate and 10 drops of sulphuric acid were added, the mixture being heated in a boiling water bath for 10 minutes, cooled, and diluted with water to about 30 ml. The mixture was extracted once with chloroform, made alkaline with ammonia and extracted again with chloroform. The residue left on evaporation of the chloroform was dissolved in about 2 ml.

of acetone and 1 to 2 drops of a 10 per cent. solution of sodium hydroxide were added; cocaine gave an intense purple colour. Amylocaine hydrochloride treated similarly gave no colour; procaine hydrochloride, a light reddish-violet colour, changing quickly through brown to greenish-yellow; benzocaine, no colour; homatropine hydrobromide, a light reddish colour; atropine sulphate, a strong violet colour. R. E. S.

Cortisone and Related 17:21-Dihydroxy-20-Ketosteroids, a Quantitative Colour Reaction for. C. C. Porter and R. H. Silber. (*J. biol. Chem.*, 1950, **185,** 201.) 17:21-Dihydroxy-20-ketosteroids react with phenylhydrazine and sulphuric acid to give a yellow colour. The steroid is dissolved in 1 ml. of methyl alcohol and is heated at 60°C. with 8 ml. of phenylhydrazine solution (65 mg./100 ml. sulphuric acid, 1·63:1) for 20 monutes. When cool the optical density is measured at 410 mμ against a reagent blank. To correct for interfering steroid compounds a second determination is made omitting the phenylhydrazine. The difference in the two readings is proportional to the quantity of 17:21-dihydroxy-20-ketosteroid in the sample. With plasma it is frequently necessary to apply an additional correction for the opalescence of the solutions. With 1 to 25 μg. of pure cortisone acetate the optical density was directly proportional to the concentration of steroid, and the method was sensitive to about 1 μg. It was possible to estimate precisely the amount of cortisone acetate in a solution containing 20 μg. each of this steroid, methyltestosterone, œstradiol and pregnenolone, in spite of the interference which these substances caused. Other interfering substances such as fructose and dehydroascorbic acid can be eliminated in the preliminary preparation of the sample. With extracts of adrenal cortex it was found difficult to dissolve out the keto-steroids. When they were added to blood and urine they could readily be extracted with chloroform, the results showing about 80 to 95 per cent. recovery. Only after prolonged administration of the compounds to rats was it possible to detect them in the urine. A. D. O.

Digitalis, Chemical Assay of. F. Neuwald. (*Arch., Pharm., Berl.*, 1950, **283,** 93.) The method of Knudson-Dresbach, based on the reduction of alkaline picrate, gives high results in the determination of digitalis glucosides. This is not due to any reducing effect of the sugar fraction, but to some other compound present in the leaves. The author has modified this method by first separating the genins, as follows: 1 g. of coarsely powdered drug is infused with 100 ml. of water at 90°C. for 15 minutes, cooled, filtered and made up to 100 ml.: 40 ml. of this solution is treated with 2 ml. of 10 per cent. solution of lead acetate, and made up to 50 ml. After a short time the mixture is filtered, and to 25 ml. of the filtrate is added 1 ml. of 10 per cent. solution of disodium phosphate. After filtration, the residue is washed well with water. To the filtrate is added 1 ml. of 0·1N hydrochloric acid, and water to about 50 ml., and the mixture is boiled gently for 30 minutes, cooled, and neutralised to litmus with ammonia. The liquid is evaporated to dryness on the water-bath, and the residue is digested successively with 10 ml., then 5 ml., and 5 ml. of chloroform, the chloroformic solutions being washed with water. The chloroform is evaporated off and the residue dissolved in 5 ml. of methyl alcohol: 5 ml. of freshly prepared alkaline picrate reagent is added and, after 30 minutes, the extinction coefficient is determined at 20°C., using filter S50. The method is standardised against pure digitoxin solution. In applying this method it was found that the chloroform-insoluble fraction gave a strong reaction, although it did

not give the Legal reaction. This fraction was very toxic, but with frogs the stoppage of the heart occurred in diastole, whereas with the heart-active glucosides and genins it stops in systole. Thus, there is in the leaf a toxic substance which is not a typical heart poison. G. M.

Emodins in Drugs, Determination of. R. Fischer and E. Buchegger. (*Pharm. Zentralh.*, 1950, **89**, 261.) The determination of total emodins in rhubarb, frangula and cascara is carried out as follows: 0·3 to 0·5 g. of the finely powered drug is heated on the water-bath, under a reflux condenser, for 10 minutes with 15 ml. of methyl alcohol, 0·05 ml. of dilute sulphuric acid and 5 to 10 drops of hydrogen peroxide (30 per cent.). The mixture is then evaporated to 2 ml. and heated under a reflux condenser with 25 ml. of chloroform. After filtering through cotton wool, the filtrate is concentrated to 10 ml. and treated with a little sodium bicarbonate and anhydrous sodium sulphate. This chloroformic solution is passed through a column containing in succession 4 g. of sodium bisulphite, 2 g. of kieselguhr and (as bottom layer) 4 g. of ammonium carbonate. The liquid passing through is passed over a second column containing 8 g. of calcium carbonate. Washing with chloroform is continued until the liquid coming out of the first column is colourless. The second column is then washed with 5 + 5 + 3 ml. of alcohol (96 per cent.). When the red colour has almost reached the bottom of the column, a few drops of potassium hydroxide solution are added to the receiver in order to show when emodin commences to come through the column, when the washing is stopped immediately. The calcium carbonate is removed from the tube, and the red band treated with hydrochloric acid and extracted with chloroform. The chloroform solution is passed through a column containing 8 g. of finely powdered calcium chloride to remove impurities, the chloroform solution is evaporated to dryness and the residue is weighed. The ammonium carbonate used for the column should be purified by extraction with boiling alcohol, and drying at 60° to 70°C. Free emodins may be determined in a similar manner by direct extraction of the drug with chloroform, while treatment with sulphuric acid alone, followed by chloroform extraction, gives free and combined emodins. Anthranols are then calculated from the difference between total emodins and free + combined emodins. For senna leaves the first stage of extraction is as described above, but if the chloroform solution has a greenish colour instead of orange brown, it must, after drying, be treated with a little sodium peroxide. Immediately after the colour has changed to brown the peroxide should be filtered off. In this case the final residue is dissolved in 5 per cent. sodium hydroxide solution and 2 per cent. ammonia solution and made up to 200 ml. The emodin is then determined colorimetrically, using chrysophan as standard, with a filter absorbing at 475 or 560 mμ. G. M.

Emulsions, Use of Chlorophyll for Breaking. I. C. Edmundson and B. J. Wilkins. (*Analyst*, 1950, **75**, 169.) During the extraction of alkaloids in alkaloidal assays of solanaceous drugs it was noticed that the emulsions became worse as the chlorophyll was extracted. Quantities ranging from a few drops to a few ml. of a 5 per cent. solution of chlorophyll in chloroform were therefore added to the emulsions. Breaking began immediately on shaking and was complete within a short time; the treatment was used with success on tinctures and extracts which contained chlorophyll. Commercial spirit-soluble chlorophyll was used, a blank determination showing it to have

no effect on the final titre. Any tendency to emulsification in the subsequent acid extractions can be prevented if the acid layers are bulked together with any emulsion and shaken, although one extra chloroform wash may be needed to remove all the chlorophyll. R. E. S.

Strychnine and Brucine, Chromatographic Separation of. R. Fischer and E. Buchegger (*Pharm. Zentralh.*, 1950, **89**, 146.) For the assay of nux vomica or semen ignatii, 2 g. of the drug is shaken for 3 hours with 60 g. of chloroform and 2 ml. of 2N sodium hydroxide. The mixture is filtered through cotton wool in a covered filter, and 30 g. of the chloroform solution is taken. This is concentrated and shaken out with 20, 15 and 15 ml. of 1 per cent. hydrochloric acid, the combined aqueous extracts being washed with 15 ml. of chloroform. The solution is made alkaline with ammonia, and the bases are shaken out into three 15 ml. quantities of chloroform. The chloroformic solution, after drying with sodium sulphate, is evaporated to dryness and the residue is taken up in 10 ml. of warm neutral trichlorethylene. The solution is passed through a column of 10 g. of alumina in a tube of 9 mm. diameter, the column being sucked dry and washed with two 5 ml. quantities of solvent. This trichlorethylene liquor is rejected. Strychnine is then eluted from the column by 75 ml. of a mixture of carbon tetrachloride containing 9 per cent. of acetone, the first 15 ml. of eluate being rejected. After removal of the solvent, the strychnine is dissolved in 10 ml. of 0·01N hydrochloric acid and determined by titration. Brucine is then eluted from the column by 25 ml. of ethyl alcohol and determined in a similar manner. G. M.

ORGANIC CHEMISTRY

***p*-Aminosalicylic acid, Manufacture of.** C. van der Stelt and W. T. Nauta. (*Pharm. Weekbl.*, 1950, **85**, 474.) In the manufacture of *p*-aminosalicyclic acid by carboxylation of *m*-aminophenol with carbon dioxide at high pressure, a certain amount of 4-amino-6 hydroxy*iso*phthalic acid is formed. This compound is only slightly soluble in water and organic solvents, and may best be identified by the melting-points of its methyl and ethyl esters, 146° to 147°C. and 140° to 141°C. respectively. The MLD50 for mice is between 2 and 2·4 g./kg. G. M.

Citric Acid, Production of, by *Aspergillus niger*. F. W. Kunstmann (*Pharm. Zentralh.*, 1950, **89**, 259.) The yield of citric acid from a glucose culture may be doubled by suitable preliminary treatment of the organism. Strains of *Aspergillus niger* should be selected by culture in acid medium (*p*H 1·6 to 1·4) at 28°C.; the spores formed are inoculated on to molasses-agar at *p*H 6·0 and 35°C.; the spores produced after 3 to 4 days are then used as inoculum. The culture solution should contain 0·01 per cent. of zinc sulphate and 0·28 per cent. of pure ferric chloride. G. M.

Phenacetin, Acet-4-chloranilide as Impurity in. J. Hald. (*Dansk Tidsskr. Farm.*, 1950, **24**, 183, 195.) A sample of phenacetin which had been found to cause methæmoglobinæmia in patients, was found to contain 18 per cent. of acet-4-chloranilide. In a number of samples examined, quantities ranging from 0·02 to 0·6 per cent. were found. Up to 1·5 per cent. does not depress the melting-point of phenacetin below the official limit. The presence of this material is due to the reaction of *p*-chloronitrobenzene (the usual

starting point for phenacetin) with sodium ethoxide being incomplete, so that the *p*-nitrophenetole contains unchanged chloronitrophenol. On reduction and acetylation this gives acet-4-chloranilide. The latter compound was actually isolated from impure specimens of phenacetin by a long process of purification, but its presence may be detected by a determination of combined chlorine in the phenacetin. For this the author prefers the method of Zacherl and Krainick, involving distillation with chromic acid and titration of the chloride with mercuric nitrate, using diphenylcarbazone as indicator. G. M.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Ayfivin and Bacitracin, Resolution of Crude Products in Similar Series of Peptides. G. G. F. Newton and E. P. Abraham. (*Biochem. J.*, 1950, **47**, 257.) Ayfivin is an antibiotic produced by a strain of *Bacillus licheniformis*. The crude material was resolved by counter-current distribution and the behaviour of its components was compared to those of bacitracin. In a solvent system composed of amyl alcohol-*n*-butyl alcohol and 0.05M phosphate buffer at *p*H 6.8 to 7. crude ayfivin resolved into at least seven components, but with a system comprised of *sec*.-butyl alcohol and dilute acetic acid the resolution of the components was very poor. Three of the components, designated A, B and C, were found to be polypeptides possessing antibacterial activity. Component A, the major constituent. was equally as active as C and four times as active as B. The distribution curve of commercial bacitracin in the first system was similar to that of partially purified ayfivin. A mixture of ayfivin A and the corresponding component of bacitracin could not be resolved. Ayfivin B and C. also behaved in a similar way to two other components of bacitracin. The active components of bacitracin and ayfivin are probably identical and it is suggested that the active components of the latter should be called bacitracin A, B and C. A. D. O.

Insulin, Infra-red Evidence of Chain Configuration in Natured and Denatured. A. Elliott, E. J. Ambrose and C. Robinson. (*Nature, Lond.*, 1950, **166**, 194.) A sample of crystalline insulin was cast from formic acid solution at high temperature and the frequency of the infra-red absorption band characteristic of the C=O peptide link measured. The curve obtained by plotting wave number against optical density was similar to that obtained with insulin denatured by boiling in dilute hydrochloric acid solution. The same material, redissolved in *m*-cresol and recast as a film, gave a curve similar to that obtained with insulin natured by precipitation from aqueous phenol. Since synthetic polypeptides cast from solution in *m*-cresol are predominantly in the folded (α) form, but when cast from solution in formic acid are in the extended (β) chain configuration, the results support the view that natured insulin consists of polypeptide chains in the same α-fold as α-synthetic polypeptide and that denaturation is accompanied by extension into the β-form. The type of α-fold enables the α-β transformation to occur without any marked change in the side-chain packing but merely by rotation of the -CO-NH- groups about bonds attached to the asymmetric carbon atom; no rotation of side-chains about the chain axis is required and the transformation could occur without breaking the disulphide linkages, which must be preserved if denaturation is to be reversible. G. R. K.

Phosphate, Inorganic, Mechanism of Absorption from Blood by Tissue Cells. G. Popják. (*Nature, Lond.*, 1950, **166,** 184.) Rabbit liver was perfused *in situ* with homologous plasma to which a small amount of carrier free inorganic $^{32}PO_4^{3-}$ had been added (2 to 5 microcuries per litre) with or without 0·01M sodium azide. The amounts of phosphorus-32 which disappeared from the perfusion fluid were measured at 2-minute intervals and the amount of inorganic phosphorus absorbed by the liver calculated from the known inorganic phosphorus (^{31}P + ^{32}P) content of the perfusion fluid. The average absorption was 14 µg./minute in the control perfusion and 3·6 µg./minute in the perfusion with azide, a difference amounting to a 74 per cent. inhibition. Since azide inhibits phosphorylation, the simplest interpretation of the results is that the transfer of inorganic phosphate across cell membranes requires phosphorylating reactions. The concentration of inorganic phosphorus in tissue cells is higher (10 to 12 mg. per cent. in the liver) than in the plasma (3 to 4 mg. per cent.) and a transfer of phosphate against the concentration gradient requires expenditure of energy. The phosphorylating reactions on the cell membrane rather than those within the cytoplasm are concerned in the transfer of phosphate from the extracellular to the intracellular phase.

G. R. K.

Pyrogens, Decrease of, on Storage. J. Dorche and M. Castaing. (*Ann. pharm. franc.*, 1950, **8,** 365.) The pyrogenic character of a solution generally decreases considerably on storage over a period of months. Those which were found not to do so all contained glucose, and it appears that in this case the pyrogenic substance was derived from the glucose and not from the water.

G. M.

Streptomycin, A New. R. G. Benedict, F. H. Stodold, O. R. Shotwell, A. M. Borud and R. A. Lindefelser. (*Science*, 1950, **122,** 77.) The streptomycin described in this paper is produced by an organism which differs from previously recorded sources. The aerial mycelia of the mould slowly change from greyish white to a flesh colour and the name of *Streptomyces griseo-carneus* n.sp. is therefore suggested for it. In certain characteristics the compound resembled the known streptomycins, but by Winston and Eigens' method, it was completely separable from mannosidostreptomycin and streptomycin in artificially prepared mixtures. Investigation of the structure suggested the name of hydroxystreptomycin. Its specific rotation (trihydrochloride) differed from both streptomycin and mannosidostreptomycin, and, on catalytic reduction, it absorbed the amount of hydrogen required to form a dihydro compound. When the trihydrochloride was assayed against *B. subtilis* it had the equivalent activity of streptomycin base 748 µg./mg. (streptomycin trihydrochloride 842 µg./mg.). A probable structure has been assigned to the compound but larger amounts of material are necessary to confirm it.

A. D. O.

Tetanus Toxoid, Concentration of. M. B. Jacobs and M. A. Behan. (*J. Amer. pharm. Ass., Sci. Ed.*, 1950, **39,** 466.) Tetanus toxoid was precipitated at *p*H 3·5 to 4·0 with trichloroacetic acid. About 800 ml. of toxoid is mixed with 80 ml. of N trichloroacetic acid and set side for 30 minutes. The solution plus precipitate was then carefully distributed in four 250-ml. centrifuge cups and centrifuged sufficiently to pack the precipitate. The supernatant liquid was drained off and the contents of all the cups was dissolved in 10 ml. of phosphate buffer (*p*H 8). The concentration was determined by Ramon's limit of flocculation test and adjusted with 0·85 per cent.

saline solution. Thiomersalate (1 in 10,000) was added as a preservative, and the toxoid was sterilised by passing it through a Berkefeld filter. Toxoid prepared in this way passed the tests for potency and limits and toxicity of the U.S. National Institutes of Health. Not more than 17 per cent of the toxoid was lost in processing, and the flocculation time was only slightly increased. Nearly all the nonspecific nitrogen was removed. A. D. O.

Tetanus Toxoid, Purification of. M. B. Jacobs. (*J. Amer. pharm. Ass., Sci. Ed.*, 1950, **39**, 469.) The purity of a concentrated toxoid may be considered from the points of view of either the ratio of the flocculation units/mg. of nitrogen (Lf/mg.N) in the purified toxoid to the Lf./mg.N of the total protein (total undialysable nitrogen) in the crude preparation, or to the Lf./mg. of total nitrogen. Using the latter ratio the author has investigated products prepared by the method previously described (*J. Amer. pharm. Ass., Sci. Ed.*, 1950, **39**, 466). It was found that, by this method and some slight modifications of it, it was possible to remove about 99 per cent. of nitrogen. The modified processes, which entailed extra washings of the precipitate or similar treatment, enabled a purer product to be obtained but resulted in greater working losses. A. D. O.

BIOCHEMICAL ANALYSIS

Acetaldehyde in Blood, Spectrophotometric Determination of. T. N. Burbridge, C. H. Hine and A. F. Schick. (*J. Lab. clin. Med.*, 1950, **35**, 983.) A method for the rapid determination of blood acetaldehyde levels is described, which has permitted as many as 48 determinations to be completed in a 5-hour period. The separation of acetaldehyde from blood is accomplished by diffusion in a Conway cell. A semicarbazide solution is used as the reactant and the resulting acetaldehyde semicarbazone is determined spectrophotometrically with a Beckman DU spectrophotometer. When a 2 ml. blood specimen is analysed, acetaldehyde in concentrations as low as 0·2 µg./ml. can be determined with an accuracy of ± 3·5 per cent. Blood samples of 0·5 to 2 ml. are adequate for the amount of acetaldehyde encountered in normal blood and the blood of patients undergoing antabuse therapy. Normal acetaldehyde blood levels as determined by this method range from 40 to 110 µg per cent. Diffusion eliminates interference from all non-volatile agents, and there is no indication of interference from the possible presence of acetone. S. L. W.

Adrenal Cortical Hormones; Analysis by Paper Partition Chromatography and Occurrence in the Urine of Normal Persons. A. Zaffaroni, R. B. Burton and E. H. Keutmann. (*Science*, 1950, **111**, 6.) The corticoids were sufficiently soluble in polar solvents and their α-ketol group was sufficiently detectable to obviate the use of Girard T reagent. The best solvent systems were benzene-formamide and toluene-propylene glycol. The paper strips were dipped in the polar solvent, the excess removed, the samples applied to the strips and the chromatograms developed by the descending method with benzene or toluene saturated with its respective polar solvent. The papers were dried and the positions of the corticoids revealed by treatment with alkaline silver nitrate (10 ml. of 0·1N silver nitrate, 10 drops of concentrated ammonium hydroxide and 5 ml. of 10 per cent. sodium hydroxide) followed after maximum colour production with a 5 per cent. sodium thiosulphate solution. As little as 10 to 15 µg. of a corticoid could be detected. Cortisone,

but no other corticoid tested, gave an intense blue colour in amounts over 15 µg. when the chromatogram was sprayed with a 0·3 per cent. solution of iodine in a 5 per cent. potassium iodide solution. The benzene-formamide system was most suitable for preliminary fractionation of complex corticoid mixtures, for the resolution of individual $C_{21}O_3$ compounds and to analyse the acetates and propionates of cortisone and 17-hydroxycorticosterone. The toluene-propylene glycol system gave wider and more rapid separation of $C_{21}O_5$ compounds. The method was applied to urine collected from 5 patients. The urines were extracted with ether at *p*H 1, and the extracts washed with dilute alkali and acid and separately analysed. The presence of cortisone and 17-hydroxycorticosterone was strongly indicated by the agreement in chromatographic behaviour of the urinary compounds and the known steroids, the similar movements of their esters, the characteristic colour reactions and the typical ultraviolet absorption curves. The normal 24-hour excretion of each was estimated to be between 20 and 40 µg. No other cortical hormone was found in appreciable amount. G. R. K.

Adrenaline in Biological Media, Micro-determination of. E. Sinodinos and R. Vuillaume. (*Bull. Soc. Chim. biol.*, 1950, **32,** 409.) A new colour reaction of adrenaline is obtained by adding to an acid solution of adrenaline, *p*-nitraniline and sodium nitrite, and making alkaline. The colour is greenish-blue, although it becomes brownish if the proportion of adrenaline is too high. The sensitivity is 1 µg. Details are as follows: to 1 ml. of adrenaline solution, containing not more than 20 µg., 0·1 ml. of saturated solution of *p*-nitraniline in N sulphuric acid, 1 drop of 1 per cent. solution of sodium nitrite and 2 drops of strong sodium hydroxide are added. The colour reaches its maximum intensity immediately, and is stable for several hours. Determination in adrenal gland: the gland is rubbed down with sand and 5 per cent. solution of trichloracetic acid and, after filtering, the determination is carried out on the filtrate. Determination in blood; the blood is deproteinised with trichloracetic acid, and the filtrate is passed through alumina at *p*H 8. After elution at *p*H 2 to 3, the reaction is applied directly. It is necessary to apply a factor to allow for the loss which occurs during these operations (the value of this factor is not stated). A similar colour is given by certain adrenaline derivatives; in the case of nor-adrenaline only 1/10 the strength of that with adrenaline. Although the presence of nor-adrenaline has been reported in adrenal gland, the amount appears to be too small to have any appreciable affect on the results of this method. G. M.

***p*-Aminosalicylic Acid, Determination of.** R. Fleury. (*Bull. Trav. Soc. Pharm. Bordeaux*, 1950, **88,** 68.) For the determination of *p*-aminosalicylic acid in pus, a volume of the latter is diluted with an equal volume of physiological salt solution. After centrifuging, the sediment is treated with saline solution until no more of the acid can be detected in the liquid. The solution is diluted from 20 to 500 times, and 5 ml. of it is treated with one drop of 10 per cent. solution of ferric chloride. The colour is then compared with a series of freshly-prepared standards containing from 0·1 to 0·033 per cent. of *p*-aminosalicylic acid. The results obtained by this method are equally as accurate as those obtained by diazotisation, and it has the advantage that it is applicable directly to acetaminosalicylic acid, the form in which *p*-aminosalicylic acid is eliminated. For determination in urine, it is only necessary to dilute the sample from 50 to 100 times, and then adjust to *p*H 3 by the addition of hydrochloric acid. When *p*-aminosalicylic acid (9 g.) is injected

into an abscess, it is eliminated completely in 36 hours. Diffusion in the abscess is very slow at first and is only complete after 8 hours. During this period urinary elimination is very low. G. M.

Benzylpenicillin, a New Method for the Spectrophotometric Determination of. H. Pénau, G. Hagemann, Y.-G. Leclére and R. Viennet. (*Ann. pharm. franc.*, 1950, **8**, 450.) Commercial penicillin salts may be assayed for benzylpenicillin by the following method. Prepare a solution of the dried material in a mixture of 95 parts of alcohol (95 per cent.) and 5 parts of water, and determine the optical density at 263·0 mμ, 280·0 mμ and 322·0 mμ. Unless the optical density at 322·0 mμ is greater than that at 280·0 mμ, in which case the substance has undergone decomposition and the result will not be valid, determine the ratio of the optical densities at 263·0 mμ and 280·0 mμ and calculate the percentage of benzylpenicillin by reference to a standard curve prepared with a chromatographically pure sample of benzylpenicillin. The determination is accurate to ±2 per cent., and avoids the use of aqueous solutions in which there is rapid deterioration and shift of absorption maxima. G. B.

Globulin and Total Protein in Cerebrospinal Fluid, Microphotelometric Determination of. H. B. Salt. (*J. Lab. clin. Med.*, 1950, **35,** 976.) A method is described for the precipitation of globulin by methyl alcohol (45 per cent.) at *p*H 6·6 in the presence of ghatti gum in 1 hour at 37°C. Albumin is not precipitated under these conditions provided its concentration is below the critical level of 125 mg./100 ml. A similar procedure is described for the precipitation of total protein by salicylsulphonic acid in the presence of ghatti gum in 5 minutes at room temperatures. Details are given (together with a sectional plan and elevation) of a photoelectric apparatus whereby the precipitated proteins, held in colloidal suspension by the gum, are determined directly by light dispersion measurements. Dispersimetric values are correlated with globulin and with total protein concentrations by means of empirically established curves. Spectral red light is normally used, but for greater sensitivity the wave-band may be changed to spectral violet. The analytical procedures described are applicable to diluted blood serum as well as to cerebrospinal fluid and give reliable results for quantities of protein from 0·1 mg. to 1·4 mg. per aliquot analysed. S. L. W.

Insulin Preparations, Commercial, A Comparison of Three Methods for the Assay of. D. M. Young, D. B. W. Reid and R. G. Romans. (*Canad. J. Res. Sect. E,* 1950, **28,** 19.) Commercial preparations including crystalline and non-crystalline insulin, protamine zinc insulin and globin insulin with zinc were assayed by three methods, (i) determining blood-sugar levels after subcutaneous injection into 32 rabbits using the "twin cross-over" design; (ii) determining the blood-sugar levels after intravenous injection into 16 rabbits using the 4 × 4 Latin square design; and (iii) determining the number convulsing within 75 minutes after subcutaneous injection into 288 mice, using a two-level quantal response design. There was satisfactory agreement between the results by the three methods. The average standard error of the result was: method (i), 13 per cent., method (ii), 10 per cent., and method (iii), 9 per cent. G. B.

Penicillin in Urine, Determination of. H. H. Pénau, E. Saïas, N. de Chezelles and D. Benoist. (*Ann. pharm. franc.*, 1950, **8,** 444.) A quantity of urine containing not less than 5 units of penicillin is adjusted

to pH 2 and extracted twice with chloroform in the presence of sufficient ammonium sulphate to depress considerably the solubility of penicillin in the aqueous phase. The chloroform solution, after decolorisation, if necessary, with charcoal, is extracted with aqueous sodium hydroxide, and the penicillin titrated iodimetrically. Concentrated urine requires a preliminary washing with chloroform at pH 4, and this does not remove an appreciable quantity of penicillin. The urinary concentration of penicillin corresponds fairly closely to the blood level, determined microbiologically, and curves relating urinary penicillin concentration with time may be used in estimating blood levels maintained with various salts of penicillin. G. B.

Vitamin D, Spectrophotometric Estimation of. H. E. Cox. (*Analyst*, 1950, **75**, 521.) The following method is recommended for the determination of vitamin D in foodstuffs. Dissolve the unsaponifiable matter in 10 ml. of *cyclo*hexane, and to 0·2 ml. of the solution add 1·8 ml. of a freshly prepared, alcohol-free reagent containing 20 per cent. w/v of antimony trichloride and 4 per cent. of acetyl chloride. If vitamin A is present, a blue colour is produced, and the size of sample is adjusted so that the colour fades in about 4 or 5 minutes. Determine the absorption at 500 mμ after 6 minutes, and calculate the vitamin D content from the datum $^{1 \text{ per cent.}}_{1 \text{ cm.}}$ = 1880 for pure vitamin D_3. If the composition of the material is known, as when the test is used for control of manufacture, a correction can be applied for the colour produced by other sterols. The test is four times more sensitive than the assay by determination of absorption at 265 mμ, and is not so susceptible to interference by other sterols and vitamin A. When there is five or more times as much vitamin A as vitamin D present, the vitamin A should be removed chromatographically, before assaying for vitamin D. G. B.

PHARMACY

NOTES AND FORMULÆ

Acetomeroctol (Merbak). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950, **143**, 814.) Acetomeroctol, 2-(acetoxymercuri)-4-(1:1:3:3-tetramethylbutyl)phenol, $C_{16}H_{24}O_3Hg$, is a white solid, m.pt. 155° to 157°C., almost insoluble in water, soluble in alcohol, ether and chloroform, and sparingly soluble in benzene. When 3 ml. of chloroform containing 0·1 g. of iodine is gradually added to a solution of 0·2 g. of acetomeroctol in 3 to 5 ml. of chloroform, the iodine colour is immediately discharged, a momentary green appears and yellow mercuric salts are precipitated. When acetomeroctol is extracted with water and filtered the filtrate gives no colour or precipitate with sodium sulphide (absence of mercuric ion); the loss in weight on drying *in vacuo* over anhydrous calcium sulphate for 24 hours is not more than 0·75 per cent. Acetomeroctol contains 43·0 to 43·2 per cent. of mercury calculated with reference to the dried substance. It is assayed by heating under a reflux condenser with monoethanolamine, separating the globule of mercury thus obtained by centrifuging, dissolving in nitric acid and estimating by titration with potassium thiocyanate. Acetomeroctol is applied locally in 0·1 per cent. solution containing 50 per cent. of alcohol and 10 per cent. of acetone as an antiseptic for the control of superficial infection. G. R. K.

Bismuth Glycolylarsanilate (Milibis). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950, **143**, 895.) Bismuth glycolylarsanilate, the product

of reaction between sodium *p-N*-glycolylarsanilate and bismuth nitrate, is an odourless, yellowish-white to flesh-coloured amorphous powder which decomposes on heating. It is very slightly soluble in water and alcohol and insoluble in benzene, chloroform and ether; a saturated aqueous solution has *p*H 2·8 to 3·5. When a solution in acidified water is boiled, cooled and treated with bromine solution, a white precipitate is obtained, which on extraction with ether yields tan-coloured crystals of 2:4:6-tribromoaniline, melting at 118° to 121°C. (presence of arsanilic acid). It contains not more than 0·5 per cent. of free arsanilate, calculated as arsanilic acid and determined by titration with sodium nitrite in ice-cold acid solution, and not more than 3 per cent. of moisture when dried at 105°C. for 24 hours. The content of arsenic is 14 to 16 per cent., of bismuth 36 to 42 per cent., and of arsanilic acid 42·2 to 44·8 per cent. Arsenic is assayed by digesting with acid, adding sodium potassium tartrate, neutralising with sodium hydroxide and titrating with iodine in the presence of sodium bicarbonate; bismuth is assayed by digesting with nitric acid, diluting, treating with diammonium phosphate and weighing the precipitate so obtained; and arsanilic acid is assayed by refluxing with hydrochloric acid, cooling in ice and titrating with sodium nitrite. Bismuth glycolylarsanilate is an amœbicide given as tablets in an average adult dose of 0·5 g. thrice daily.

G. R. K.

Choline Bicarbonate. (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950, **143,** 814.) Choline bicarbonate, (2-hydroxyethyl)trimethylammonium bicarbonate, $CH_2OH.CH_2.N(CH_3)_3.HCO_3$ is prepared by passing carbon dioxide into a solution of choline until the *p*H falls below 9, concentrating, again passing in carbon dioxide until the *p*H falls to 8·5 to 8·9, and evaporating *in vacuo*. It is a very hygroscopic, white solid with an amine-like odour, very soluble in water and alcohol, and slightly soluble in benzene, chloroform and ether; a 10 per cent. aqueous solution has *p*H 8·5. It gives a white curdy precipitate with phosphotungstic acid and an emerald green colour with cobaltous chloride and potassium ferrocyanide. When treated with hydrochloric acid, choline bicarbonate evolves carbon dioxide; the resulting solution after gentle heating and diluting gives no colour or precipitate with hydrogen sulphide; ash not more than 0·05 per cent. It contains 98 to 105 per cent. of choline bicarbonate and is assayed by treating an aqueous solution with ammonium reineckate, filtering, washing the precipitate with water and alcohol, drying by suction, dissolving in acetone and measuring the absorption at 5260Å with a spectrophotometer; the content of choline is obtained from a standard curve obtained by similar determinations on a series of solutions of choline chloride reference standard U.S.P. Choline bicarbonate is recognised for clinical trial as an adjunct in the treatment of fatty infiltration of the liver; the total daily dose is 8 g. or more.

G. R. K.

Isopropylarterenol Hydrochloride (Isuprel Hydrochloride). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950, **144,** 238.) Isopropylarterenol hydrochloride is α-(*iso*propylaminomethyl)protocatechuyl alcohol hydrochloride or 1-(3′ : 4′-dihydroxyphenyl)-2-*iso*propylaminoethanol hydrochloride, $C_6H_3(OH)_2.CHOH.CH_2.NH.CH(CH_3)_2,HCl$. It is a white, odourless, slightly bitter, nonhygroscopic, crystalline solid, m.pt. 166° to 172°C., soluble in water and alcohol and very slightly soluble in benzene and ether; aqueous solutions become pink on standing. A 1 per cent. aqueous solution

is clear and colourless and has pH 4·5 to 5·5. It may be distinguished from amphetamine, ephedrine, methamphetamine, naphazoline, phenylpropanolamine, phenylpropylmethylamine and tuaminoheptane by the dark brown to black colour which develops when it is added to a 10 per cent. solution of ammonium molybdate in sulphuric acid; the addition of ferric chloride to an aqueous solution gives an intense green colour which becomes olive-green on standing (distinction from hydroxyamphetamine and phenylephrine, which give a purple colour). Isopropylarterenol hydrochloride loses not more than 1·0 per cent. of its weight when dried to constant weight over phosphorus pentoxide; ash, not more than 0·2 per cent. It contains 5·40 to 5·80 per cent. of nitrogen (determined by semi-micro Kjeldahl) and 14·1 to 14·8 per cent. of hydrogen chloride (determined by adding silver nitrate and weighing the silver chloride precipitated). A 0·0004 per cent. solution in water exhibits an ultra-violet absorption maximum at 2800 Å, and has $E_{1\,cm.}^{1\,per\,cent.}$ of 113 ± 3, equivalent to 97 to 103 per cent. of isopropylarterenol hydrochloride. Tablets and solution are assayed by measuring the transmission at 2800 Å and calculating the content of isopropylarterenol hydrochloride form a standard curve. Isopropylarterenol hydrochloride is a sympathomimetic amine closely related in its action to adrenaline and noradrenaline. It is effective in the treatment of mild and moderately severe asthma. When inhaled it exerts a mild expectorant action. It is administered sublingually in a dose of 10 to 15 mg. or by inhalation in a dose of not more than 0·5 ml. of a 0·5 per cent. solution. G. R. K.

Isopropylarterenol Sulphate (Isonorin Sulphate). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950, **144,** 239.) Isopropylarterenol sulphate is α-(*iso*propylaminomethyl)protocatechuyl alcohol sulphate or 1-(3′:4′-dihydroxyphenyl-2-*iso*propylaminoethanol sulphate. $(C_6H_3(OH)_2.CHOH.CH_2.NH.CH(CH_3)_2)_2.H_2SO_4$. It is a white, odourless, slightly bitter, hygroscopic, crystalline solid, m.pt. 118° to 122°C., freely soluble in water, slightly soluble in alcohol and very slightly soluble in benzene and ether; aqueous solutions become pink on standing. A 1 per cent. aqueous solution is clear and colourless, and has pH 3·5 to 4·5. Isopropylarterenol sulphate responds to the colour tests for isopropylarterenol hydrochloride. When dried to constant weight: over phosphorus pentoxide it loses not more than 7·0 per cent. of its weight: ash, not more than 0·3 per cent. It contains 5·20 to 5·60 per cent. of nitrogen, determined by semi-micro Kjeldahl. A 0·0004 per cent. aqueous solution exhibits an ultraviolet absorption maximum at 2800Å and has $E_{1\,cm.}^{1\,per\,cent.}$ 106 ± 3, equivalent to 97 to 103 per cent. of isopropylarterenol sulphate.
G. R. K.

Mercaptomerin Sodium (Thiomerin Sodium). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950, **143,** 895.) Mercaptomerin sodium is the disodium salt of N(γ-carboxymethylmercaptomercuri-β-methoxy)propyl-camphoramic acid and occurs as a white, hygroscopic solid which decomposes when heated to 150° to 155°C. It is freely soluble in water, soluble in alcohol and almost insoluble in ether, benzene and chloroform. When a solution is treated with sodium acetate and cobalt nitrate followed by potassium iodide, a deep orange colour is produced. The presence of allyl-camphoramic acid is shown by treating an aqueous solution with sodium sulphide and an excess of hydrochloric acid, boiling and filtering; the white crystals obtained on concentrating the filtrate melt between 171° and 173°C. Mercaptomerin sodium contains 3·60 to 3·98 per cent. of

sodium, 31·4 to 34·8 per cent. of mercury and 14·4 to 15·6 per cent. of mercaptoacetic acid; the molecular ratio of mercaptoacetic acid to mercury is not less than one. When dried over phosphorus pentoxide at 100°C. for 3 hours it loses not more than 2 per cent. of its weight. The content of mercury is determined by electrolysing an aqueous solution containing sodium sulphide for twenty-four hours at a current density of 0·5 amp. using a rotating platinum cathode, and measuring the increase in weight of the cathode. Mercaptoacetic acid is assayed by adding potassium iodide and glacial acetic acid and titrating with potassium iodate. Mercaptomerin sodium is a mercurial diuretic and is administered subcutaneously as a 14 per cent. aqueous solution in a dose of 0·5 to 2 ml. G. R. K.

Methoxyphenamine Hydrochloride (Orthoxine Hydrochloride). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950, **143**, 897.) Methoxyphenamine hydrochloride is β-(*o*-methoxyphenyl)*iso*propylmethylamine hydrochloride, $CH_3O.C_6H_4.CH_2.CH(CH_3).NH(CH_3),HCl$. It occurs as an odourless, bitter, white, crystalline powder, melting at 124° to 128°C.; it is freely soluble in water, alcohol and chloroform and slightly soluble in benzene and ether, the *p*H of a 5 per cent. solution in water being 5·3 to 5·7. When distilled with hydriodic acid, methyl iodide is obtained in the distillate; when the residual solution in the flask is cooled to 0°C., treated with diazotised *p*-nitroaniline and made alkaline, a bright orange-red precipitate forms (presence of hydroxyphenyl group). Methoxyphenamine hydrochloride loses not more than 0·5 per cent. of its weight when dried *in vacuo* over phosphorus pentoxide for 24 hours; ash, not more than 0·5 per cent. It contains 98 to 102 per cent. of methoxyphenamine hydrochloride (determined by the Kjeldahl method) and 16·25 to 16·65 per cent. of chlorine. Methoxyphenamine hydrochloride is a sympathomimetic agent and is administered as tablets in doses of 50 to 100 mg. every 3 or 4 hours. G. R. K.

Tripelennamine Citrate (Pyribenzamine Citrate). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950, **142**, 569.) Tripelennamine citrate, *N*:*N*-dimethyl-*N'*-benzyl-*N'*(α-pyridyl)ethylenediamine citrate, is a bitter, white, crystalline powder, m.pt. 106° to 110°C. It is freely soluble in water, and alcohol, very slightly soluble in ether and almost insoluble in benzene and chloroform; a 1 per cent. solution has *p*H 4·25. It gives a flocculent pink precipitate with ammonium reineckate, and a dipicrate melting between 184° and 186°C. When treated with sulphuric acid it turns brown but does not dissolve (distinction from the hydrochloride). When dried *in vacuo* over phosphorus pentoxide for 24 hours, it loses not more than 0·5 per cent. of its weight; ash not more than 0·3 per cent. It contains 98 to 102 per cent. of tripelennamine citrate and is assayed by weighing the dipicrate obtained by treatment with trinitrophenol. Tripelennamine citrate is a histamine antagonist and has the same action as the hydrochloride; it is, however, more palatable. The average dose is 75 mg. 4 times a day. G. R. K.

PHARMACOGNOSY

Eucalypt Kinos, Chromatographic Analysis of. W. E. Hillis. (*Nature, Lond.*, 1950, **166**, 195.) Butyl alcohol-acetic acid-water (40-10-50 per cent.) gave poor resolution of the components of the eucalypt kinos, and phenol-water, although more suitable did not give sharp resolution. The most suitable

solvents were (a) a mixture of phenol and 2N acetic acid containing 0·3 per cent. of sodium chloride; (b) a mixture of equal volumes of phenol and an aqueous solution 2N with respect to both acetic and hydrochloric acids; and (c) ethyl alcohol-benzene-water (40-20-40 per cent.). Solvents (a) and (b) gave comparable R_f values but (b) was more suitable for old samples of kino. Solvent (c) resolved only those compounds which had a high R_f value in the phenol solvents. The kino of *E. calophylla* R. Br. (marri) was resolved into at least 12 components (6 fluorescent and 6 detected by Tollen's reagent), that of *E. corymbosa* Sm. into at least 13 (5 fluorescent, 7 by Tollen's reagent, 1 by both), and that of *E. goniocalyx* F. v. M. into at least 11 (4 fluorescent, 5 by Tollen's reagent, 1 by both and 1 carbohydrate). Aromadendrin had an R_f value of 0·79 and showed a green fluorescence in ultraviolet light. G. R. K.

Frangula Extract, Chromatographic Examination of. P. F. Jørgensen. (*Dansk Tidsskr. Farm.*, 1950, **24,** 111.) By a series of fractionations on various columns, a number of fractions were obtained from liquid extract of frangula. These are summarised in the table below, with their probable modes of origin.

	Compound	Formed from	Reaction
1.	Anthrone-anthranol glucoside complex		——
2.	Anthraquinone glucoside complex A	1	Hydrolysis and oxidation
3.	Anthraquinone glucoside complex B	1	Hydrolysis and oxidation
4.	Chrysophanic acid anthrone + frangula emodinanthrone monomethyl ether	1	Hydrolysis
5.	Frangula emodinanthrone	1	Hydrolysis
6.	Difrangulin	3	Hydrolysis
7.	Frangulin	6	Hydrolysis
8.	Condensation product of partly oxidised frangula emodinanthrone	5	Oxidation
9.	Chrysophanic acid + frangula emodin monomethyl ether	4	Oxidation
		2	Hydrolysis
		3	Hydrolysis
10.	Frangula emodin	2	Hydrolysis
		8	Oxidation
		5	Oxidation
		7	Hydrolysis

G. M.

PHARMACOLOGY AND THERAPEUTICS

Adrenocorticotrophic Hormone in Poliomyelitis. L. L. Coriell, A. C. Siegel, C. D. Cook, L. Murphy and J. S. Stokes. (*J. Amer. med. Ass.*, 1950, **142,** 1279.) This is a report of a clinical investigation undertaken to obtain answers to the following questions: (1) Is the "alarm reaction" evoked by poliomyelitis? (2) What is the physiological action of the drug in poliomyelitis? (3) Does the drug modify the course of the disease when administered in the early stages? A total of 70 patients with poliomyelitis was studied 35 receiving the drug and 35 a placebo (isotonic sodium chloride solution). The drug was given intramuscularly at 6-hourly

intervals for 4 or 5 days. The results showed (1) that the "alarm reaction" is mobilised in poliomyelitis as shown by the eosinophil response; (2) that in these patients the drug produced a physiological effect as evidenced by a further depression of the eosinophil count and by decreased excretion of 17-ketosteroids; (3) that there was no demonstrable effect on the treated as compared with the untreated group when evaluated on the basis of temperature response, paralysis, progression of paralysis, or early residual effects. Statistical analysis showed that the drug has no beneficial or obvious deleterious effect on the course of poliomyelitis when treatment is begun after the onset of symptoms. S. L. W.

Adrenocorticotrophic Hormone Therapy in Inflammatory Diseases of the Eye. J. A. Olson, E. H. Steffenson, R. R. Margulis, R. W. Smith and E. L. Whitney. (*J. Amer. med. Ass.*, 1950, **142,** 1276.) Of 7 patients with inflammatory eye diseases 4 had acute plastic iritis and 2 had keratitis and anterior uveal tract involvement, 1 of the 2 with secondary glaucoma, 1 patient had a recent chorioretinitis with absolute scotoma. The drug was given intramuscularly at intervals of either 4 or 6 hours in dosages of 10 to 20 mg. Total dosages ranged from 185 to 432 mg. and treatment was continued over periods of from 3 to 13 days. In all patients attempts were made to avoid early relapses by withdrawing the drug slowly after the last 24 to 36 hours of treatment. All the patients responded abruptly and favourably to the treatment. Symptomatic relief and distinct objective changes were achieved as early as the second hour in 1 patient and by the 4th hour in the other patients. 2 of the patients with acute plastic iritis were "cured" and showed no evidence of relapse in 5 and 7 weeks. Glycosuria in 2 patients and auricular fibrillation in 1 patient were observed during the period of administration. S. L. W.

Antimycin A, an Antibiotic with Insecticidal and Miticidal Properties. G. S. Kido and E. Spyhalski. (*Science*, 1950, **112,** 172.) Antimycin is derived from an unidentified species of *Streptomyces* and is a potent fungicide. It is an optically active nitrogenous phenol of the molecular formula $C_{28}H_{40}O_9N_2$. For antimycin to exert its poisonous effect against insects it must be ingested. Certain insects such as the German cockroach and the larva of the webbing clothes moth (*Tineola bisselliella,* (Hum.)) are immune; larvaæ of *Attagenus perceus,* (Oliv.) will not feed on fabrics treated with it. In one-hundredth of the concentration, antimycin A will afford the same protection for fabrics as the normal amount of sodium aluminium silicofluoride against larvæ of the black carpet beetle. It is more effective than methoxychlor in controlling Mexican bean larvæ and di(*p*-chlorophenyl)-methyl carbinol against red spider mite. A. D. O.

Aureomycin, Treatment of Pneumococcal Pneumonia with. H. F. Dowling, M. H. Lepper, H. H. Hussey, E. R. Caldwell and H. W. Spies. (*J. lab. clin. Med.,* 1950, **35,** 215.) 174 cases of pneumococcal pneumonia were treated with aureomycin. 250 mg. was administered orally every 3 hours, or 500 mg. every 6 hours until temperature fell and remained normal for 48 to 72 hours. Temperature fell more rapidly than in penicillin-treated patients. The fatality rate in cases of typed pneumococcal pneumonia was 1·4 per cent., compared with 5·2 per cent. in penicillin-treated cases, but this difference is not statistically significant because of the relatively small number of patients treated witth aureomycin. G. B.

Bacitracin, Treatment of Amœbiasis with. H. Most, J. W. Miller, E. B. Grossman and N. Conan. (*J. Amer. med. Ass.*, 1950, **143,** 792.) 51 patients with amœbiasis varying in severity from an asymptomatic condition to fulminating dysentery were treated with bacitracin given as tablets. The total daily dose varied from 40,000 to 120,000 units and treatment extended for from 5 to 20 days. In 34 patients there was apparent parasitic cure after the first course of treatment, but the remainder relapsed; of these, 9 received a second course and of these, 6 again relapsed. Extension of therapy beyond 10 days or increase in dose above 80,000 units did not significantly enhance the probability of cure. In 8 patients who were moderately or severely ill, the clinical response was notable in that the dysentery was brought under control within a few days, the amœbæ disappeared from the stools and surface lesions, and complete healing occurred in from four to fourteen days. In 3 patients, *E. histolytica* reappeared in the stools 5, 8 and 350 days after the last dose of bacitracin, but were unaccompanied by clinical activity. The toxicity of bacitracin is negligible and the drug is apparently little absorbed. G. R. K.

Chloramphenicol, Hæmopoietic Changes during Administration of. I. F. Volini, I. Greenspan, L. Ehrlich, J. H. Gonner, O. Felsenfeld and S. O. Schwartz. (*J. Amer. med. Ass.*, 1950, **142,** 1333.) In 3 patients, 2 with typhoid and 1 with brucellosis, profound blood and marrow changes were observed during chloramphenicol therapy. These changes consisted of a precipitous fall in the total leucocyte count, which occurred by the 7th day in 1 case and continued as long as the drug was administered. The leucopenia resulted primarily from a decrease in the number of granulocytes in the marrow due to a maturation arrest without significant alteration in the monocytes or lymphocytes. The total dosages employed were 53 g. in 18 days in one case, 53 g. in 19 days in the second case, and 26 g. in 9 days in the third case. The treatment was remarkably effective in controlling the infection in all the patients; an immediate precipitous rise in granulocytes and leucocytes followed discontinuance of the chloramphenicol therapy. The toxic manifestations of chloramphenicol in man will have to be studied more extensively before it can be considered an absolutely safe therapeutic agent, particularly when large total dosages are employed over relatively short time periods. S. L. W.

Chloramphenicol, Placental Transfer of. W. C. Scott and R. F. Warner. (*J. Amer. med. Ass.*, 1950, **142,** 1331.) In order to determine whether or not there was placental transfer of chloramphenicol 12 normal pregnant patients were given the drug during term labour in doses of 2 g. orally at 2-hour intervals until delivery occurred. At the time of delivery 10 ml. of maternal venous blood was drawn and 10 ml. of fœtal blood was collected from the umbilical cord. Colorimetric determinations revealed that 10 of the 12 had a therapeutically effective serum concentration in fœtal cord blood within 71 minutes after initial dosage. This level was maintained for at least 135 minutes and by 2-hourly repetition of the dose could be maintained as long as 625 minutes. No evidence of toxicity was exhibited by any of the patients or infants. S. L. W.

Dicoumarol Labelled with Carbon-14, Tracer Experiments in Mammals with. J. W. T. Spinks and L. B. Jaques. (*Nature, Lond.*, 1950, **166,** 184.) Fourteen mice were each given 0·25 mg. of labelled dicoumarol intravenously and sacrificed at various times after injection. No significant radioacivity

was detected in the lungs, kidney or tissues in general, but great activity was found in the liver, gall bladder, fæces and urine; 80 per cent. of the activity disappeared from the blood in the first hour following injection. A series of rabbits similarly treated with 10 mg. of labelled dicoumarol gave a similar distribution pattern, with a rapid disappearance of dicoumarol from the blood and a rapid increase of activity in the liver; it was shown that the activity in the liver was essentially all due to unchanged dicoumarol, whereas although a large percentage of activity was recovered in the urine at the end of the experiment, none of this was due to dicoumarol. In the mice, the dicoumarol remained in the liver for one day and the prothrombin time remained high for 3 days, whereas in the rabbits the corresponding periods were 7 days and 8 days respectively. When mice were injected with the labelled dicoumarol together with vitamin K, activity appeared in the liver immediately, but disappeared much more quickly than from the livers of animals not receiving the vitamin. The results show that the liver is the target organ for the action of dicoumarol and suggest that the period of time during which it remains in the liver is related to its effectiveness in interfering with the formation of prothrombin. G. R. K.

Mephenesin in Infantile Cerebral Palsy. C. H. Frantz. (*J. Amer. med. Ass.*, 1950, **143**, 424.) Mephenesin (3-ortho-toloxy-1 : 2-propanediol) given by mouth over a 7-month period was well tolerated by 23 out of a test group of 27 children with infantile cerebral palsy. 4 doses of mephenesin a day were given, 20 minutes before meals and 30 minutes before retiring. Children under 10 received 1 g. a day initially as 4 tablets of 250 mg., and children over 10 were started at 1·5 g. daily. From 5 to 7 days the dose was increased by adding 1 (or more) tablet per meal. Thereafter, monthly, the dosage was increased to tolerance. The average doses over a prolonged period were between 0·75 and 3 g. Vertigo, nausea, vomiting, irritability and listlessness were undesirable side effects noted in children receiving doses up to 2 g. Vertigo was in most cases easily controlled by a change in dosage. No undesirable results were shown in blood and urine studies. Of 16 athetoid children 14 showed beneficial effects. Children with tension athetosis seem to give a more consistently favourable response than other groups. Children with spasticity and rigidity did not respond satisfactorily. S. L. W.

Morphine, Pharmacological Properties of New Derivatives of. P. Chabrier, R. Giuducelli and K. Kristensson. (*C. R. Acad. Sci., Paris,* 1950, **231**, 289.) The two new compounds described are the dibromomethylate of morpholylethylmorphine, and dihydromorpholylethylmorphine. The dibromomethylate shows a toxicity 30 times as great as morpholylethylmorphine itself. Thus toxicity of the bases, which decreases in the order codeine, morphine, morpholylethylmorphine, is reversed in the methyl bromide compounds. This fact is the more remarkable as codeine is 7·5 times more toxic than morpholylethylmorphine. With all three, bromomethylation has the effect of reducing considerably, in intensity and duration, the inhibitory respiratory action; decreasing their convulsive power; and causing curarising properties. The latter is, for rabbits, about 10 times as great with the morpholylethylmorphine compound as for morphine and codeine. G. M.

Phenosulfazole (Darvisul) in Acute Poliomyelitis. M. J. Fox and E. Z. Hornberger. (*J. Amer. med. Ass.*, 1950, **143**, 535.) 29 patients who

had bulbar and spinobulbar forms of poliomyelitis were treated with phenosulfazole, *N*-(2-thiazolyl)-phenol sulphonamide, in a dose of 400 mg./kg. of bodyweight daily; the drug was given both intravenously and orally. Among this group the mortality rate was 34·5 per cent. During the same period 19 patients did not receive the drug and showed a mortality rate of 42·1 per cent. There appeared to be no alteration in the length of temperature elevation or the length of hospitalisation in the group receiving the drug, and instances of drug toxicity were not noted. The authors conclude that phenosulfazole has little to offer in the treatment of poliomyelitis, since the mortality rate in both the treated and untreated groups fell within the expected range for a severe epidemic. S. L. W.

Pituitary Adrenocorticotrophic Hormone Therapy in Ophthalmological Conditions. D. M. Gordon and J. M. McLean. (*J. Amer. med. Ass.*, 1950, **142**, 1271.) Pituitary adrenocorticotrophic hormone was given to 6 patients with the following diseases:—severe corneal œdema (corneal dystrophy), secondary glaucoma, chronic iridocyclitis, retinitis pigmentosa and acute choroiditis. The usual dosage was 25 mg. intramuscularly 3 or 4 times daily. The duration of treatment was controlled by the short supply of the drug and the response of the patient; in no instance was treatment continued for longer than 9 days. The circulating eosinophil count offers a good index of the dosage. The response of the patients with iridocyclitis and choroiditis was dramatic, that of the patient with retinitis pigmentosa apparently temporarily beneficial. The other 2 patients failed to show any favourable response to short courses of treatment. No adverse systemic effects were noted other than temporary hypertension, glycosuria and mild abdominal distension. Every patient showed a definite fall in circulating eosinophils. S. L. W.

Pituitary Posterior Lobe Extracts, Assay of. G. A. Stewart. (*Analyst*, 1950, **75**, 542.) For oxytoxic activity, various modifications of the *in vitro* guinea-pig uterus method are in use, with different assay patterns, and different concentrations of magnesium and calcium in the Ringer's solution. The most precise isolated uterus method appears to be that using the non-pregnant rat uterus in a modified Locke's solution having one half the usual calcium and one quarter the usual glucose concentration, with a 4-point design. The depression of blood pressure in fowls anæsthetised with phenobarbitone sodium is an effect of the oxytocic hormone. This is the basis of a quick and reliable *in vivo* assay, and the vasopressor hormone does not affect the results appreciably unless the ratio vasopressin/oxytocin is greater than 10. Vasopressor activity may be estimated by recording the blood pressure in spinal cats or anæsthetised dogs, and antidiuretic activity may be assayed by delay in excretion of water in rats, or inhibition of diuresis in dogs. It should not be assumed that commercial pituitary extracts all contain the hormones in the same relative proportions. Separate tests for oxytocic, vasopressor and antidiuretic activity should be made. G. B.

Pyrogens, Action of, in Rabbits. J. Dorche and M. Castaing. (*Ann. pharm. franc.*, 1950, **8**, 353.) Methods, based on leucocytosis in rabbits, have been proposed as a test for pyrogens. The authors find that pyrogenic solutions, when injected intravenously, produce a leucopenia of short duration followed by hyperleucocytosis affecting the polynuclear leucocytes. On account of the instability of the blood of the rabbit, it does

not appear that any test of this kind would be more sensitive than that of hyperthermy. Untreated rabbits rarely show a percentage of polynuclear leucocytes greater than 50, and any higher value, if confirmed 3 or 4 hours after the injection, is generally parallel with the hyperthermy. G. M.

Pyrogens, French Official Test for. J. Dorche, G. Bouthier, M. T. Ardiet and M. Castaing. (*Ann. pharm. franc.*, 1950, **8**, 358.) In using the French official test, it is essential to take certain precautions. Some animals show an abnormal reaction, small ones tending to be hyposensitive and large ones hypersensitive. The sensitivity of the animals should be tested by a suspension of typhoid bacilli (200 million /ml.), heated at 115°C. for one hour. This suspension retains its activity for a long period. A dose of 0·5ml./kg. of body weight should produce a rise of temperature of 0·9 to 1·5°C. Alternatively a freshly prepared and sterilised dilution (1:20) of T.A.B. vaccine may be used. Any type of thermometer may be used, but it should be inserted to a depth of 75 mm. Strongly hypotonic solutions should be made isotonic. In 30 negative tests, the range was within ±0·3°C. in 84 per cent. of the cases. The official limit of 0·6°C. may therefore be considered reasonable. G. M.

Terramycin in the Treatment of Venereal Disease. F. D. Hendricks, A. B. Greaves, S. Olansky, S. R. Taggart, C. N. Lewis, G. S. Landman, G. R. MacDonald, and H. Welch. (*J. Amer. med. Ass.*, 1950, **143**, 4.) Terramycin at the proper dosage effects a satisfactory cure rate in the treatment of gonorrhœa, though the dose required for cure is somewhat higher than has been necessary with chloramphenicol. From 1 to 2 g. of terramycin hydrochloride given by mouth in divided doses gives a cure rate of 80 to 100 per cent. (based on treatment of 73 cases), while single doses of 750 mg. of chloramphenicol give similar cure rates. Clinical healing of lesions of both syphilis and granuloma inguinale occurs promptly with terramycin hydrochloride given orally in doses of 60 mg./kg. of bodyweight daily. S. L. W.

Terramycin Hydrochloride, Clinical Observations on the Use of. E. Q. King, C. N. Lewis, H. Welch, E. A. Clark, J. B. Johnson, J. B. Lyons, R. B. Scott and P. B. Cornely. (*J. Amer. med. Ass.*, 1950. **143,** 1.) Terramycin hydrochloride was administered to 30 patients with various types of infection; these included penumococcal pneumonias, urinary tract infections due to *Escherichia coli* and *Aerobacter aerogenes*, whooping cough bacteræmia due to *Salmonella,* pneumonitis and lung abcess with mixed bacterial infections. The drug was given by mouth according to the following dosage schedules. In patients of 14 years and older without urinary tract infections 750 mg. every 6 hours; with urinary tract infections 500 mg. every 6 hours; in children of 9 years and younger 500 mg. every 4 hours. Assayable amounts of terramycin were found in the blood and urine within 1 hour and for 5 hours after administration of 750 mg. of the drug by mouth. There was a good response to the treatment in the majority of cases and the results obtained in urinary tract infections were promising. There was a low incidence of side reactions most of which were mild and subsided with continued treatment; they included nausea, abdominal pain, slight headache, mild transient erythema, vomiting and diarrhœa. In an occasional instance severe gastro-intestinal distress, including diarrhœa, may necessitate withdrawal of the drug. S. L. W.

BOOK REVIEWS

CELL PHYSIOLOGY AND PHARMACOLOGY, by *J. F. Danielli.* Pp. viii + 153 (including 21 illustrations, 3 plates and 22 tables). Cleaver-Hume Press Ltd., London. 1950. 24s.

A knowledge of the function of cells and the effects of drugs thereon is fundamental to the understanding of the complexities of drug action. Yet to-day we must admit that we know very little of the biological reactions occurring within the cell and the manner in which drugs affect them. Much of our information consists of hypotheses and data from results with an inherent degree of inaccuracy. Pharmacology is a young science which is rapidly developing and, while we are gaining an insight into many problems, as we progress we become more acutely aware of the complexities of biological systems. We may compare our present knowledge with that of the chemist prior to the elucidation of atomic structure. Reading Dr. Danielli's book one is soon aware of the lack of progress in this field since the classical publication of the late Professor Clark on the " Mode of Action of Drugs on Cells " in 1933. It is to be hoped that the main purpose of the book, to stimulate research in the cellular action of drugs, is fulfilled.

The book itself is based upon a series of lectures given at University College, London, and is chiefly concerned with the physico-chemical aspects of the cell and its reactions to drugs. It is divided into six chapters dealing with the cell as a physico-chemical unit; the actions of drugs on surfaces, membrane permeability and enzymes; the actions of narcotics and the response of cells on the biological level. It assumes, from the reader, a reasonable knowledge of physical chemistry and for the student a more elementary account of the complex systems and formulæ involved would appear desirable. The book is a valuable contribution to biological research on drug action and can be profitably read by chemists who may then more fully appreciate the limitations of the biological worker.

G. F. SOMERS.

LETTERS TO THE EDITOR

Correction.

CONVERSION OF VITAMIN B_{12b} INTO VITAMIN B_{12}

BY B. ELLIS, V. PETROW, G. H. BEAVEN, E. R. HOLIDAY AND E. A. JOHNSON.

This Journal, 1950, **2**, 735,

Lines 10 and 17, *for* B_{12} *read* B_{12b}

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