REVIEW ARTICLE

BLOOD AND BLOOD PRODUCTS

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The use of blood and blood derivatives in medicine and surgery has greatly increased in this century, though it was the investigations of workers in the 17th century which first suggested that transfusion, rather than blood-letting, might be of value in the therapy of some conditions. Blood transfusion as practised before 1900 was fraught with dangers, which were largely overcome when Landsteiner[1] demonstrated the existence of blood groups. The introduction of effective anticoagulants, and finally the drying and preserving of plasma and blood products, enabled blood and plasma to be stored. This saved the tedious procedure of calling up a donor for each separate transfusion, and direct transfusion from donor to recipient.

It is well known that human whole blood and plasma are most valuable in the treatment of shock, burns, and certain diseases where there is a depletion of one or more of the plasma components. Pooled and convalescent plasma and serum have also been used in the prophylaxis or modification of some infectious diseases.

Whole Blood and Concentrated Red Blood Cells

The collection and storage of whole blood for transfusion purposes was contemplated and practised in a small way during the First World War. Rous and Turner[2] investigated the preservation of living red blood cells *in vitro*. They found that these cells could be washed most satisfactorily using Locke's solution containing 1/4 to 1/8 per cent. of gelatin. The addition of the gelatin protected the cells from breakdown, even when shaken, though it was necessary to transfer the washed cells to a fluid containing no gelatin, if they were to be kept without hæmolysing. The best results were obtained by using mixtures of Locke's solution and isotonic solutions of sugars in water. In such solutions they found that the cells assumed a spherical shape. Using rabbits as experimental animals, these workers were able to show that transfusions of " kept cells " were useful in replacing lost blood, and that the animal was able eventually to dispose of them without harmful results[3].

This led Robertson[4], in 1918, to use stored blood in the treatment of wound shock on the battlefield, and he obtained excellent results, even with cells that had been kept for as long as 21 days. However, it was not until the Spanish Civil War, of 1937-9, that the use of stored blood was fully exploited, and at the advent of the Second World War an elaborate Blood Bank system was developed in this country, which is

still in operation to-day. It is controlled by the National Blood Transfusion Service, under the Ministry of Health, and consists of 2 London and 11 Regional Centres in England and Wales, with Centres in Scotland and Northern Ireland.

Donors are bled from the median cubital vein directly into sterile vessels containing a suitable anticoagulant solution. The standard anticoagulant now used consists of 100 ml. of 2 per cent. disodium citrate solution and 20 ml. of a 15 per cent. glucose solution. This is sterilised in the transfusion bottle, to which 420 ml. of blood are eventually added. This citrated blood is then stored at a temperature of + 3°C., and distributed to the hospitals, where it may be used for transfusion at any time up to 21 days after it was taken. The blood after storage for 21 days is considered unfit for transfusion. The fragility of the red cells increases, and they may hæmolyse.

Mainwaring, Aylward and Wilkinson[5] have also found that after withdrawal of blood from the body there is an increase of plasma potassium and plasma inorganic phosphate, and because of the possibility of these substances producing toxic reactions they recommend that the plasma be separated from the cells as soon as possible. It is now possible to preserve the plasma from stored blood by drying it from the frozen state.

Whole blood is most valuable in the treatment of shock caused by hæmorrhage, as in surgery, and it has also proved useful in cases of intestinal obstruction with circulatory failure of the shock type, and in acute generalised peritonitis.

Concentrated red blood cells have been used in the treatment of anæmia, and McQuaide and Mollison[6] report fewer reactions with these than with whole stored blood. The cells may be washed with saline, and preserved after so washing for about 48 hours.

Whole Dried Plasma

The plasma from time-expired human citrated blood is collected into 10 donor pools, constituted so that to each 9 bottles of Group A, 1 bottle of Group B, or AB is included to remove isoantibodies. It is then syphoned, in 400 ml. quantities, into transfusion bottles. These minute bottles are stored at + 2°C., and then frozen, by spinning them on a vertical axis at about 1,000 revs. at − 25°C., in the current of air from a fan. In this way a cone is forced down through the liquid, spreading the plasma evenly round the inside periphery of the bottle, where it is supercooled, and then rapidly freezes. It is dried from the frozen state, first by vacuum and finally over phosphorus pentoxide, and gives a satisfactory and rapidly soluble product[7]. Bacteriological tests for sterility are carried out at each step and all infected bottles discarded.

Thus plasma, which otherwise would have had to be discarded as unfit for use, may now be preserved in a dried state and reconstituted with pyrogen-free distilled water as it is required. In this way plasma can be stored for periods up to 5 years, and hence is always available in an emergency, or when there is delay or difficulty in obtaining fresh

blood. The dried plasma may be transfused to a patient of any blood group. This is of great importance when the time lost in the sampling and typing of a patient's blood may result in a deterioration of his condition, and even when a transfusion of whole blood is indicated the interim use of reconstituted plasma is often beneficial in tiding the patient over this period of delay. Human serum also may be preserved by freeze drying.

Liquid Plasma and Serum

The preservation of liquid plasma and serum has presented many difficulties, largely due to the fact that these liquids are normally rather opaque and tend to form a sediment on standing, particularly when shaken, thus making it impossible to distinguish between sterile and non-sterile material. Aseptic conditions are always adhered to during the collection and preparation of plasma and serum for transfusion purposes, but it is essential to be able to detect any infection that may inadvertently occur.

At first it was thought that Seitz filtration would remove any bacteria, and by clarifying the liquids enable them to be stored satisfactorily. It was found that plasma and serum contain unstable lipoid substances and in the case of plasma the difficulties of preparation are further enhanced by the fact that filtration, due to the activation of prothrombin by the magnesium of the asbestos of the filter pad and the subsequent conversion of the fibrinogen to fibrin, caused clots and shreds to appear in the filtered plasma. Bushby and Whitby[8] suggested a method of overcoming this post-filtration clotting by alkalinisation of the plasma prior to filtration, and then readjusting the *p*H with carbon dioxide. This was not entirely satisfactory, and it seemed that the complete removal of fibrinogen was the only answer to the problem.

Several workers have suggested methods for accomplishing this. Clegg and Dible[9] prepared a transfusion material by recalcifying citrated plasma. The fibrin clot was broken up by shaking with glass beads, and the serum was then sterilised by passing it through a Seitz filter. They found that this material gave no skin reaction when injected intradermally, and provided a satisfactory transfusion fluid.

Reid and Bick[10] recommend the production of serum by recalcification of centrifuged plasma, as they were able to demonstrate the presence of muscle-stimulating and vasoconstrictor substances in serum formed by the clotting of whole blood. They thought that the transfusion reactions occasionally seen after transfusion of serum were due to these. Toxic symptoms following the transfusion of serum have also been reported by some other workers, but the evidence is by no means clear.

Maizels[11] has described a procedure for removing the fibrinogen from plasma by adsorption on kaolin. The process involves five steps, the kaolin is autoclaved in bottles, plasma is added, shaken and the kaolin allowed to settle. The plasma is then removed, passed through a paper pulp filter, and finally sterilized by passage through an antibacterial filter pad. This material gave excellent clinical results, and Maizels claims

that its use was so particularly free from reactions that it has been suggested that kaolin may also remove pyrogens or other toxic substances sometimes present in plasma.

It was found that after a single absorption this fluid was still not stable in the liquid state, and the technique was found most useful for plasma drying, although Maizels reported that a second and third treatment with kaolin produces a more stable material.

The use of alcohol and ether at low temperatures has proved the most satisfactory means of obtaining a stable liquid for transfusion. This was first suggested by Hardy and Gardiner[12] who added serum or plasma to a large volume of alcohol or acetone at − 8°C., and then washed the precipitate with ether. Bick[13] describes a modification of the method, and precipitates serum proteins with a mixture of alcohol and ether at − 12° to − 14°C. McFarlane[14] has claimed that the unstable lipoids are removed from serum by freezing it with ether below − 25°C. Electrophoresis of serum treated 3 times in this manner showed that the concentration of beta globulin had been reduced, and when the process was applied to plasma, the fibrinogen had been completely removed. The resultant fluid was crystal clear, and had good keeping qualities.

We may therefore summarise by saying that at present whole blood, washed concentrated red cells, liquid filtered plasma and reconstituted dried plasma are available for transfusion in this country. Whole blood is used for surgical shock and the replacement of blood lost. Concentrated red cells are used in the treatment of anæmia, and plasma and reconstituted plasma in emergency treatment, when the delay in finding a suitable blood group might be dangerous. Plasma is most valuable in the treatment of burns[15] where there is a fluid loss into the tissues and a rapid and extensive loss of protein accompanied by hæmoconcentration. Transfusion with plasma will reduce this hæmoconcentration and restore the circulating blood volume.

The Fractionation of Plasma

In the treatment of some conditions it will be realised that all the constituents of plasma are not equally effective, and indeed whole plasma may be less effective and less economical than the use of the separated components. Among the constituents of plasma that have thus far been concentrated are the antibodies against infectious diseases, two of the components of complement, the anti-A and anti-B isohæmagglutinins and Rh antibodes, hypertensinogen, thyrotropin, fibrinogen and thrombin, and the various groups of electrophoretically defined globulins and albumins.

It is most important that any process of plasma fractionation shall not irreversibly alter the plasma components, as such proteins would be foreign to the human body, and their introduction into the circulation may result in the production of undesirable reactions. The methods of separation that have been developed depend upon differences in the physico-chemical properties of the plasma proteins. It is possible to isolate

proteins by electrophoresis, but only on a small scale, so this method finds most useful application in the control and improvement of the bulk fractionation methods. The solubility of proteins in aqueous solution is determined by such variables as *p*H, salt concentration, temperature, total protein concentration, and in some procedures, solvent concentration.

Earlier methods of protein precipitation depended on varying the salt concentration—the so-called "salting-out" procedures. However, the necessity of afterwards removing the precipitating salt by dialysis makes it difficult to maintain sterility and, as interactions betwen proteins and salts appear to be more specific at low salt concentrations, the effects tend to be masked at the high concentrations needed for salting out. Both these disadvantages are overcome by using a solvent to precipitate the protein. A solvent like alcohol or ether can be added under sterile conditions and is, indeed, often bacteriostatic in itself. Its volatility ensures its complete removal during the freeze-drying of the final product, and the process can be carried out at low electrolytic concentrations where effects are more specific. Solvent precipitations must be performed at low temperatures, between 0° and −10°C., to prevent denaturation and subsequent loss of biological activity of the protein. Methyl alcohol. ethyl alcohol, acetone, dioxane and ether have all been used in the fractionation of proteins from aqueous solution, but of these ethyl alcohol and ether are now in most common use.

Electrophoretic studies show that normal human plasma is composed of albumin 55 per cent., alpha globulin 14 per cent., beta globulin 13·5 per cent., gamma globulin 11 per cent. and fibrinogen 6·5 per cent. In the production of protein fractions for clinical use, biological activity is more important than physico-chemical purity, though the fractions prepared do correspond fairly closely with the electrophoretic components.

The Fractionation of Plasma by Ethyl Alcohol

The development of the ethyl alcohol method of plasma fractionation by Cohn[16] and his co-workers in Harvard originated from the war-time need for a process for the production of concentrated human albumin for transfusion. By varying *p*H, ionic strength, and alcohol concentration Cohn prepared 5 main fractions from normal pooled human plasma

TABLE I

Fraction	*p*H.	Ethyl alcohol per cent.	Temp. °C.	Ionic Concentration
I	7·4	8	−3	0·14
II & III	6·8	25	−5	0·09
IV	5·8	40	−5	0·09
V	4·8	40	−5	0·11

(Table I). These fractions, when examined electrophoretically, showed the following compositions, in g. of protein/litre of plasma (Table II).

TABLE II

Fraction	Plasma	I	II & III	IV	V	VI
Albumin	33·2	0·2	0·7	1·0	29·0	0·3
α Globulin ...	8·4	0·2	1·8	5·4	0·6	0·3
β Globulin ...	7·8	0·8	6·2	3·1	—	—
γ Globulin ...	6·6	0·5	6·0	0·2	—	—
Fibrinogen ...	4·3	2·6	1·6	—	—	—

Preparation of Plasma Products by the Ether Fractionation Process

In this country plasma fractionation is now carried out using ether as a precipitating agent. McFarlane[14] in 1942 found that the extraction of plasma and serum with ether at low temperatures produced a stable transfusion fluid, and electrophoresis of the plasma treated in this way showed that beta globulin had been reduced and fibrinogen removed. This led Kekwick, Mackay and Record[17] to investigate the possibility of using ether to prepare biologically active fibrinogen and thrombin for clinical use. The process has now been developed to give gamma globulin also[18].

Fresh citrated plasma is clarified by passing it through a paper pulp filter and then, at the normal *p*H and ionic strength of plasma, 11 vols. per cent. of ether are added slowly, with stirring, at a temperature of 0° to −0·5°C. The resulting precipitate, which consists largely of fibrinogen, is allowed to settle overnight at 0°C. It is centrifuged and may be dissolved in citrate saline for conversion to fibrin foam, or washed with ether citrate saline, dissolved, and freeze-dried as fibrinogen.

To prepare fibrin foam, the crude fibrinogen solution is whipped to a froth by a spinning metal disc, thrombin is injected, and the material is poured into metal trays, where it is allowed to set, and then dried from the frozen state. It is finally baked and dispensed in pieces of convenient size.

Prothrombin is separated from the supernatant liquid after the fibrinogen precipitation by adjusting the *p*H to 5·35 at 0°C. The resulting precipitate is washed twice with distilled water, dissolved in citrate saline, and converted to thrombin at *p*H 7·0 by the addition of thromboplastin and Ca ions. After filtration the thrombin solution is freeze-dried in quantities to give 50, 250, and 500 units per ampoule. The supernatant liquid from the prothrombin separation is now diluted with 3 volumes of

sterile distilled water, and the beta and gamma globulins precipitated at an ether concentration of 18·5 vols. per cent., pH 5·75 and temperature – 3°C. The mixed precipitate is suspended in distilled water, and dissolved in buffer, and the beta globulins separated at pH 5·0, ionic strength 0·01, and an ether concentration of 9 vols. per cent., at 0°C. The gamma globulins which remain in solution are then precipitated by bringing the ether concentration up to 18 vols. per cent. at pH 6·70, and temperature – 3·5°C. This precipitate is dissolved and dried from the frozen state in quantities to give about 250 mg. per ampoule. In the freeze-drying of small volumes of protein solutions the ampoules are loaded directly into the drying chamber, and frozen by centrifugal vacuum spin freezing[7] so that the freezing and drying processes are combined in the one operation.

Clinical Uses of Plasma Products

Fibrinogen.—The fibrinogen-containing fraction of plasma is largely used, in conjunction with thrombin, for the promotion of fibrin clots in certain surgical procedures. The great advantage of human fibrin is that it may be introduced into the body and left *in situ* without fear of its behaving as an antigen and producing undesirable reactions. In America it is also used for the preparation of fibrin clots, fibrin films and fibrinogen plastics. These are made in a great variety of shapes, sizes and mechanical properties, and treated in such a way as to modify their susceptibility to attack by proteolytic enzymes, as well as their rate of absorption in living tissues. Morrison and Singer[19] find that untreated fibrin films persist in tissue for a period up to 5 days, but fully treated films have been known to last for more than 80 days. With films of extended persistence time, an encapsulation occurs between 10 and 30 days after implantation. Bailey and Ingraham[20] have tested fibrin films for their suitability in the repair of dural defects and the prevention of meningocerebral adhesions, and they claim that they seem superior to metal foil, preserved dura, fascialata, rubber, gutta-percha or amniotic membrane. In the formation of plastics[21] the fibrinogen is not clotted with thrombin, or even separated from the accompanying globulins, but Cohn's entire Fraction I is subjected to an irreversible moulding treatment. Fibrinogen and thrombin have also been used for the surface treatment of burns[22,23] for nerve suturing, skin grafting, and in coagulum pyelolithotomy. In the latter case, the fibrinogen solution is injected into the renal pelvis, and converted to fibrin by the subsequent injection of thrombin. The calculi are enmeshed and removed with the coagulum. Dees[24] has reported that this fibrinogen coagulum has several advantages in the operative removal of renal calculi, as it ensures the removal of all free stones, it avoids fragmentation of the stones during removal, trauma to the kidney is reduced to a minimum and complete surgical mobilisation of the kidney may be unnecessary.

The use of fibrinogen and thrombin in skin grafting dispenses with the need for pressure dressings, and it has been found that the grafts vascularise with great rapidity, and the tendency to bronze pigmentation seems to be reduced. Cronkite, Lozner and Deaver[25] report several cases of skin grafting in which fibrinogen and thrombin were used,

and they emphasise that its great advantage is the time saved, due to quick control of hæmorrhage, fewer sutures and simplified dressings. Michael and Abbott[26] recommend the use of fibrinogen in reconstructive surgery, especially neurosurgery and tendon repair work, and Hawn, Bering, Bailey and Armstrong[22] have reported its use in the surface treatment of burns. Prepared fibrin film may be applied to the burnt area by means of freshly formed fibrin, and Macfarlane[23] found this gave satisfactory results.

It has been found that the fibrinogen fraction also contains enough of the antihæmophilic factor of normal plasma to make it most useful in preventing or arresting bleeding in hæmophiliacs, particularly when it is necessary to subject them to surgical treatment.

The existence of a substance in normal plasma which was effective in accelerating the coagulation of hæmophilic blood was noticed in 1937 by Patek and Taylor[27], and since then considerable work has been done in this field. Lewis and co-workers[28] carried out an *in vitro* and *in vivo* comparison of normal and hæmophilic blood plasma, and their derived plasma protein fractions, and they found the antihæmophilic factor was present in Cohn's Fractions I, II, III and IV—I of normal plasma. However, it is Fraction I, or the fibrinogen fraction of the ether process which is generally used in the clinical treatment of hæmophilia. Minot *et al.*[29] have tested the antihæmophilic activity of Cohn's Fraction I *in vivo,* and they report that the injection into a patient of an active globulin fraction had no influence on the effectiveness of subsequent injections of the material, though Laurence and Craddock[30] report the case of two hæmophiliacs who became refractory to further transfusion or injection of Fraction I.

Van Creveld and Mastenbroek[31] studied the effect on hæmophiliacs of a plasma fraction containing fibrinogen 82 per cent., albumin 2·3 per cent., gamma globulin 4·27 per cent., and 11·2 per cent. of " ill defined " globulins migrating in the alpha and beta region, and they found that a 2 per cent. solution of this protein mixture had a marked coagulation promoting effect *in vitro* and *in vivo*. Other clinical tests have shown that 10 ml. of a 2 per cent. solution of the fibrinogen fraction reduces the clotting time of a hæmophiliac's blood to within the normal range for about 48 hours. The material is equally effective when injected intravenously or intramuscularly.

Thrombin

Although thrombin solutions are usually used in conjunction with fibrinogen or fibrin foam, this protein has also been found valuable as a local hæmostatic agent in various surgical procedures. Tidrick, Seyers and Warren[32] used it in the control of bleeding from bone, the gall bladder and appendical bed, and following pyloro-plasty, mastectomy and biopsies. Experience using thrombin with and without soluble cellulose for local hæmostasis have been reported by Cronkite, Deaver and Lozner[33].

Fibrin Foam

Fibrin foam is a valuable hæmostatic agent in neurosurgical procedures, and as with the other fibrin products already mentioned, its great advantage is that it can be left *in situ* without exciting injurious tissue reaction. In this respect, it is superior to muscle. It is often used in conjunction with thrombin solution, in which it is soaked before being applied to the bleeding surface. The first record of the use of fibrin for hæmostasis was by Grey[34] who tried tampons made of animal and human fibrin, and later Harvey[35] used fibrin paper and films in surgery.

Bailey and Ingraham[36] report clinical and patholcgical studies on the use of fibrin foam as a hæmostatic in neurosurgery. They find it of great assistance in controlling continual oozing and even more vigorous bleeding from large venous channels, such as the dural sinuses and cerebral veins, but it is seldom effective in the arresting of bleeding from large arteries. It has proved of value in general surgery, and some workers have reported success in controlling hæmorrhage from the cut surface of liver and kidney and in prostatectomies Its usefulness has also been demonstrated in certain dental operations, particularly in hæmophiliacs.

Gamma Globulin

It has been shown that the antibodies of human plasma are largely concentrated in the gamma globulin, and so this fraction has found wide use in the prophylaxis and treatment of various diseases. Of these the most important is measles in children and many clinical tests have been carried out to ascertain the effectiveness of gamma globulin in the prevention and attenuation of this disease.

In America, using Cohn's Fraction II, Stokes, Maris and Gellis[37] found that in a controlled group of cases with exposure within the family, gamma globulin from normal pooled adult plasma gave a rate of 71 per cent. protection, 27 per cent. modification and only 2 per cent. failure among 62 inoculated children, whereas of 46 uninoculated controls only 7 per cent. failed to contract measles. Of the remainder, 4 per cent. had the disease in a mild form, and 89 per cent. developed measles of average severity. Ordman, Jennings and Janeway[38] also carried out clinical tests, which further proved the value of this plasma fraction in the treatment of measles.

In this country, tests were performed to compare the effect of human serum gamma globulin, prepared in America by Cohn's process, with that of convalescent measles serum[39]. The gamma globulin was found to be about twice as potent as the convalescent serum. Apart from this increased potency, gamma globulin has a great advantage over serum in that there is always a risk of the latter transmitting homologous serum jaundice, whereas there has been no recorded instance of this disease following many thousands of injections of immune globulin. Using the ether fractionation process, gamma globulin is now produced in Great Britain, and clinical tests are proving most satisfactory. Several workers have investigated the effect of the gamma globulin-containing fraction on

other diseases. It has been claimed to be useful in the prophylaxis of infective hepatitis,[40] and Adams and Smith[41] have reported some success against upper respiratory infections.

A small number of patients suffering from mumps were treated with gamma globulin by Candel[42], and this appeared to reduce the incidence of orchitis as compared with untreated controls. This has been further borne out by Gellis, McGuiness and Peters[43] who administered gamma globulin, prepared from mumps convalescent serum, on the first day of the disease. Stokes[44], while emphasising the need for early diagnostic criteria of infection also found evidence that gamma globulin gave favourable results in several other diseases in man.

Albumin

During the war, it was the need for concentrated human albumin for clinical use that led to the development of Cohn's[16] method of plasma fractionation in America, and he was able to produce a precipitate that was shown to be 98 to 100 per cent. albumin by electrophoretic analysis. This material was used by the armed forces for the treatment of shock, œdema and hypoproteinæmia.

Albumin has been shown to have at least two known functions: it maintains the colloidal osmotic pressure of the blood, and plays a role in the nutrition of the tissues. Although it comprises only about 60 per cent. of the plasma proteins, it is responsible for nearly 80 per cent. of the colloidal osmotic pressure of plasma and blood, and so is the constituent of plasma that is most effective in the maintenance of blood volume. Scatchard, Batcheldar and Brown[45] have shown that each g. of albumin holds about 18 ml. of fluid in the blood stream, and therefore has an effect equivalent to about 20 ml. of citrated pooled plasma. In this respect it has proved most valuable in the treatment of shock, as it increases the blood volume without causing a fall in plasma concentration, as is the case when saline is used. However, it is recommended by Cournand *et al.*[46] that, because of the persistence of acute anæmia in many cases after albumin therapy, whole blood should be given subsequently when available.

Janeway *et al.*[47] have studied the use of albumin in the treatment of hypoproteinæmia. They find that albumin is usually the deficient plasma protein in hypoproteinæmia, and so favour its use, rather than whole plasma, in the therapy of this condition.

Salt-poor albumin has also been found to be of value in the treatment of nephrosis [48], and cirrhosis of the liver[49].

The Isohæmagglutinins

The A and B isohæmagglutinins which appear to be associated with beta globulin have been purified and concentrated from pools of plasma of suitable blood groups. High titre sera are required for accurate blood group testing, and methods of concentration make available the agglutinins from plasma whose titre is initially too low. Enders[50] reports that Cohn's Fractions II and III have isohæmagglutinin activity.

Plasma Substitutes

The difficulties associated with the collection and storage of plasma. and its dangers as a transmitter of disease, have led to a search for a material of non-human origin, which could be used as a plasma substitute. The main function of such a substitute would be to restore the circulating blood volume till the patient could replace the plasma protein lost, though it could never be more than a second best when blood rather than plasma had been lost.

The ideal blood substitute should possess certain properties. It must exert the same colloid osmotic pressure as whole blood, be readily sterilisable, non-toxic, and non-antigenic, and absolutely stable as a liquid at normal temperatures. Also it should show no deterioration on shaking, and thus be readily transportable. Probably most important of all, it should be only slowly destroyed in the body, and its elimination must be rapid and complete.

As a result of experience during the 1914-18 war, Bayliss[51] in 1919, tried the colloids soluble starch, dextrin and gelatin as blood substitutes, but these proved unsatisfactory. However, he found that a 6 per cent. solution of gum arabic in 0·9 per cent. sodium chloride had most of the desired properties, and this was introduced for general use. Various workers have since criticised the use of gum arabic on the grounds that it is stored in various organs, particularly the liver, and that it interferes with liver function. Similar storage phenomena have also been attributed to some other possible colloids for infusion, such as pectin, polyvinyl alcohol and methylcellulose.

During the 1939-45 war, the Germans introduced Periston[52], a synthetic colloid, polyvinyl pyrolidene, and they claimed this was well tolerated by the body, its action lasted for 2 days, and it was eventually excreted in 3 to 4 weeks. However, Bull and his colleagues doubt that molecules of the vinyl polymers which are large enough to be retained by the glomeruli can be metabolised by the body, and they suggest that some form of storage must take place here too.

Recently, in Sweden, " Dextran Ph " has been introduced and widely used as a plasma substitute[53]. This is a 6 per cent. solution of the polydispersoid glucose polymer dextran, in which most of the molecules have been hydrolytically given a molecular weight conforming to that of albumin. This material has certain theoretical advantages over the other non-protein colloids, because being free from acidic radicals it is not likely to form storage complexes, and it can be hydrolysed into glucose by acids and certain living organisms, suggesting that the living body may also be able to metabolize it slowly. Some tests have been carried out in this country both with the Swedish Dextran, and with material produced experimentally in Britain, and a comprehensive report has been published[54].

It is claimed that dextran is well retained in the circulation, and can maintain the osmotic pressure till its place is taken by plasma proteins. However, it is desirable to prepare dextran with a defined range of molecular size, because if the molecular weight is insufficiently reduced during

acid hydrolysis, renal damage may result. On the other hand, its value as a substitute for plasma depends on the proportion of molecules of a size which will not pass the glomerular filter.

The fate of dextran in the body is still in doubt, though serological methods can detect it in the lymph glands and spleen when it is no longer apparent in the plasma. Further investigations on this most important aspect must be done before dextran can be unreservedly recommended for widespread use, though work up to date has shown that in all other respects it appears to be a most useful plasma substitute. It has also been claimed that gelatin, when produced under rigidly controlled conditions, may be useful as a substitute for plasma.

Summary

The Blood Transfusion Service, organised as an emergency measure by the Medical Research Council in 1939, has now become an integral part of the National Health Service in England. Blood Transfusion Laboratories, under the Regional Hospital Boards, have been established in London and in the Provinces to collect blood, to carry out research and to provide specialised services in connection with blood transfusion.

A central Blood Group Reference Laboratory prepares and distributes grouping serum, and Units of the Medical Research Council have been set up to study problems associated with blood groups and blood transfusion. A combined Medical Research Council and Lister Institute Unit has been responsible for carrying out the research on plasma fractionation and for the routine preparation of plasma fractions and drying of plasma.

In the field of plasma fractionation the American workers have made many important advances in the theoretical and practical field, and their procedures have been developed commercially. In this country the emphasis has been laid on the production of those fractions most needed by clinicians, and because of the difficulty of getting equipment the scale of operations has been much smaller. The scheme is sponsored, and the expenses of the routine production are paid by the Ministry of Health, through the Medical Research Council. The collection of the necessary blood is arranged and carried out by the National Blood Transfusion Service. As the blood is a gift, the plasma fractionation products are not sold in this country, and in order to make sure that they are not wasted they are issued to clinicians through the Ministry of Health and may only be obtained by application to the Ministry. At present the use of fibrinogen is restricted to skin and nerve grafting, and the treatment of hæmophilia. Fibrin foam is used for brain and lung surgery in conjunction with thrombin, and gamma globulin has been successfully used in measles prophylaxis, in controlling the spread of the disease in hospitals, and other closed communities. At the same time, at the Lister Institute, research work to improve the different procedures goes on all the time, side by side with investigations into more fundamental problems.

References

1. Landsteiner. *Wien. klin. Wschr.*. 1901. **14,** 1132.
2. Rous and Turner, *J. exp. Med.*, 1916, **23,** 219.
3. Rous and Wilson, *J. Amer. med. Ass.*, 1918, **70,** 219.
4. Robertson, *Brit. med. J.*, 1918, **1,** 691.
5. Mainwaring, Aylward and Wilkinson, *Lancet,* 1940, **239,** 385.
6. MacQuaide and Mollison, *Brit. med. J.*, 1940, **2,** 555.
7. Greaves, *Spec. Rep. Ser. med. Res. Counc., Lond.,* 1946, No. 258.
8. Bushby and Whitby, *J.R. Army med. Cps.*, 1942, **78,** 255.
9. Clegg and Dible, *Lancet,* 1940, **239,** 294.
10. Reid and Bick, *Aust. J. exp. Biol. med. Sci.*, 1942, **20,** 33.
11. Maizels, *Lancet,* 1944, **247,** 205.
12. Hardy and Gardiner, *J. Physiol.*, 1910, **40,** LXVIII.
13. Bick, *Med. J. Aust.*, 1943, **2,** 227.
14. McFarlane, *Nature,* 1942, **149,** 39.
15. Black, *Proc. R. Soc. Med.*, 1940, **34,** 54.
16. Cohn, *J. Amer. chem. Soc.*, 1940, **62,** 3396.
17. Kekwick, Mackay and Record, *Nature,* 1946. **157,** 629.
18. Kekwick and Mackay, *Proc. Int. Conf. Biochem., Camb.*, 1949.
19. Morrison and Singer, *J. clin. Invest.*, 1944, **23,** 573.
20. Bailey and Ingraham, *ibid.*, 1944, **23,** 597.
21. Ferry and Morrison, *ibid.*, 1944, **23,** 566.
22. Hawn, Bering, Bailey and Armstrong, *ibid.*, 1944, **23,** 580.
23. Macfarlane, *Brit. med. J.*, 1943, **2,** 541.
24. Dees, *J. clin. Invest.*, 1944, **23,** 576.
25. Cronkite, Lozner and Deaver, *J. Amer. med. Ass.*, 1944, **124,** 976.
26. Michael and Abbott, *J. Amer. med. Ass.*, 1943, **123,** 279.
27. Patek and Taylor, *J. clin. Invest.*, 1937, **16,** 113.
28. Lewis, Davidson, Minot, Soulier, Tagnon and Taylor, *ibid.*, 1946, **25,** 869.
29. Minot, Davidson, Lewis, Tagnon and Taylor, *ibid.*, 1945, **24.** 704
30. Laurence and Craddock, *Blood,* 1947, **2,** 505.
31. van Creveld and Mastenbroek, *Nature,* 1946, **158,** 447.
32. Tidrick, Seegers and Warren. *Surgery,* 1943. **14,** 191.
33. Cronkite, Deaver and Lozner, *War med.*, 1944, **5,** 80.
34. Grey, *Surg. Gynec. Obstet.*, 1915, **21,** 452.
35. Harvey, *Boston med. surg. J.*, 1916, **174,** 658.
36. Bailey and Ingraham, *J. clin. Invest.*. 1944, **23,** 591.
37. Stokes, Maris and Gellis, *ibid.*, 1944, **23,** 531.
38. Ordman, Jennings and Janeway, *ibid.*, 1944, **23,** 541.
39. Report of a Sub-committee to the Blood Transfusion Research Committee of the Medical Research Council, *Lancet,* 1948, **255,** 41.
40. Stokes, *Ann. intern. Med.*, 1947, **26,** 353.
41. Adams and Smith, *Proc. Soc. exp. Biol., N.Y.,* 1946, **63,** 446.
42. Candel, *Milit. Surg.*, 1946, **99,** 199.
43. Gellis, McGuiness and Peters, *Amer. J. med. Sci.*, 1945, **210,** 661.
44. Stokes, *Yale J. Biol. Med.*, 1944, **16,** 415.
45. Scatchard, Batcheldar and Brown, *J. clin. Invest.*, 1944, **23,** 458.
46. Cournand, Noble, Breed, Lanson, Baldwin, Pinchot and Richards, *ibid.*, 1944, **23,** 491.
47. Janeway, Gibson, Woodruff, Heyl, Bailey and Nerhouse, *ibid.*, 1944, **23,** 465.
48. Thorn, Armstrong, Davenport, Woodruff and Tyler, *ibid.*. 1945, **24,** 802.
49. Thorn, Armstrong and Davenport, *ibid.*, 1946, **25,** 304.
50. Enders, *ibid.*, 1944, **23,** 510.
51. Bayliss, *Spec. Rep. Ser. med. Res. Counc., Lond.,* 1919, No. 25.
52. Hecht and Weese, *Munch. med. Wschr.*, 1943, **1,** 11.
53. Gunnar Thorsen, *Lancet,* 1949, **256,** 132.
54. Rosenquist, *Acta chir. Scand.*, 1947, **95,** suppl. 124.
55. Bull, Ricketts, Squire, Maycock, Spooner, Mollison and Paterson, *Lancet,* 1949, **256,** 134.

THE STRUCTURE OF THE FLOWER OF *HYOSCYAMUS NIGER* LINN.

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Introduction

The flowering tops of *Hyoscyamus niger* have been included in the British Pharmacopœia since 1864 as part of the official drug except during the period 1914 to 1932. Although the British Pharmacopœia, 1948, provides a brief description of the gross morphology of the flowers, it gives only scanty details of the histology. A more complete description of the gross morphology of the flower is given by Bentley and Trimen in " Medicinal Plants,"[1] and Moll and Janssonius in their " Botanical Pen Portraits "[2] give an account of the histology of the flower, which is not illustrated by drawings. The histology of the seed appears with drawings in the Anatomischer Atlas of Berg[3] and in that of Tschirch and Oesterle[4].

It was decided therefore to prepare adequate illustrations of the histological characters; this made it necessary to reinvestigate the histology of the flower. This has been done and new descriptions are given to explain the details of the drawings. A careful investigation of this kind was also required for the purpose of making a comparative study of the flowers of other closely related plants used as drugs. The gross morphology has also been reviewed as an introduction to the histological work. In the course of this work, certain details not hitherto recorded have been noted and incorporated in the new descriptions now given.

Material

For the purpose of this work, 4 samples of the biennial plant, cultivated during different years and in different places, were examined to provide material as representative of various habitats as possible. The following is the list of samples used:

1. Flowers cultivated at Long Melford, 1923.
2. Flowers from plants grown at Mill Hill, July, 1938.
3. Flowers and fruit from plants grown at Mill Hill, September, 1945.
4. Flowers from plants grown at Mill Hill, collected in June and July, 1949.

Gross Morphology

The inflorescence is described by Bentley and Trimen[1] as a two-ranked, unilateral raceme, whereas Sachs[5] describes the inflorescence as a scorpioid cyme and Tschirch and Oesterle[4] refer to it as cymose. The coiled inflorescence elongates and straightens out as the fruits develop, forming an axis about 10 cm. long, carrying about 10 to 20 sessile or very shortly

stalked flowers, each very slightly displaced from the axil of a large, leafy spreading bract; the flowers being arranged in two vertical rows on the axis with the oldest flowers at the base.

The flowers are hermaphrodite and slightly irregular.

Calyx : (Fig.1, A). *Sepals* 5, gamosepalous, inferior, forming a somewhat urceolate green calyx, covered with long clammy trichomes, abundant on the outer surface of the basal half of the tube; *tube* 4 to 15 mm. long; *lobes* triangular, 2 to 5 mm. long, slightly spreading and each terminating in a sharp apical spine; *æstivation* free: calyx persistent in the fruit.

Corolla : (Figs. 1, A and 4, A). *Petals* 5, alternating with the sepals, gamopetalous, campanulate-infundibuliform, forming a narrow *tube* about 10 to 25 mm. long; *lobes* 5, slightly unequal, rounded, 2 to 4 mm. long, inner surface velvety, colour yellow with purple veins, merging into

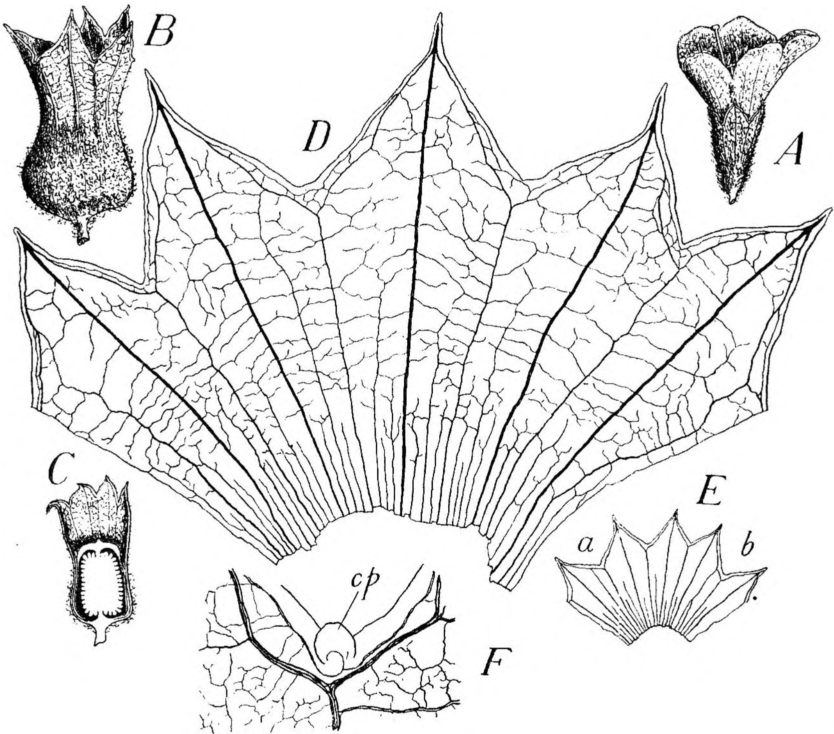

FIG. 1.—*Hyoscyamus niger* Linn., A, Flower (× 1). B, Fruit (× 1). C, longitudinal section through a partly mature fruit (× 1). D, Calyx, spread out, showing the 5 lobes and the venation (× 4). E, Calyx showing absence of fusion between the lateral veins leading to the sinuses *a* and *b* (× 1). F, details of venation at the apex of a sinus showing an early stage in the development of a cusp (× 12). *cp*, cusp.

a general bluish-green colouration at the lower part of the tube which is white at the base; trichomes frequent on the outer surface of the lobes, absent on the inner surface, rare on the tube; *æstivation* imbricate; corolla fugaceous.

Andrœcium : (Fig. 4, A). *Stamens* 5, alternating with the corolla lobes,

epipetalous, the free parts of the filament about 10 to 14 mm. long, the part adherent to the corolla being about 5 to 10 mm. long and covered with abundant trichomes. The 5 filaments which are gently curved away from the petals, vary in length and in the degree of adnation to the corolla. The length of the adherent portion varies inversely as the length of the free portion. *Anthers,* deep purple, dorsifixed at a point slightly lower than the middle, slightly curved with the convex surface inwards; dehiscence introrse along two longitudinal lateral splits before the flower is fully opened.

Gynæcium : (Fig. 7, A). *Carpels* 2, syncarpous, superior. *Ovary* (Fig. 1, C) sphæro-conical, about 3 to 5 mm. in length and 2 to 3 mm. in diameter at the widest part; bilocular, with an external equatorial groove corresponding to the line of dehiscence of the fruit, becoming more marked as the ovary matures, placentation axile, ovules numerous. *Style,* arising from the apex of the ovary, about 2 cm. long, curving in the upper part which is purple, towards the anterior petal. It terminates in a fleshy, rounded, capitate *stigma* (Fig. 7, B.), which has a funnel-shaped hollow in the centre, extended on either side as a shallow groove reaching almost to the margin.

Fruit : (Fig. 1, B). The pyxis or fruit proper is surrounded by the enlarged, persistent and markedly urceolate calyx, which is from 20 to 27 mm. long, most commonly 25 mm., the sepals being stiffer than in the flower, the inner surface glossy, the outer surface hairy. The spine at the apex of each lobe becomes more pronounced and a small cusp develops at the apex of each sinus between the lobes (Fig. 1, F). The lower $\frac{3}{4}$th of the pyxis is thin walled, but the lid, which separates by circumscissile dehiscence, is thicker and woody. The fruit contains numerous small, brown, somewhat reniform seeds.

Histology of the Calyx

Outer or lower epidermis : The cells of the outer epidermis vary along the length of the sepal. On the lobes, the cells have very wavy anticlinal walls except over the veins, the margin and the tips, where they are axially elongated with less wavy and sometimes nearly straight anticlinal walls (Figs. 2, A and B). Further down the calyx the cells have progressively less wavy anticlinal walls and become smaller and thicker walled until, at the base, they are small, sub-rectangular and thick walled (Fig. 2, D). *Dimensions :* On the lobes, L and T = 15 to **70** to 125 μ and R = 11 to **21** to 53 μ*; over the vein, however, L might be as much as 400 μ. At the base, L and T = 6 to **21** to 53 μ and R = 17 to 18 μ. The odd subsidiary cell adjoining the stomata in the cruciferous arrangement may be as small as L and T = 6 × 15 μ. The cells under the larger trichomes are less wavy, sometimes straight walled and larger than the neighbouring cells, occasionally measuring as much as 75 × 90 μ. Flowers from plants cultivated during a dry

* R, T and L indicate the measurements made in the radial, tangential and longitudinal directions respectively; the use of these symbols is suggested by Moll and Janssonius in their " Botanical Pen Portraits."[2] In cases where some cells in a given tissue show elongation in the longitudinal and others in the tangential direction, readings for L and T have been combined.

season were found smaller in size with the cellular dimensions correspondingly smaller. Typical cruciferous (anisocytic)[6] *stomata* occur and are usually slightly raised above the surface of the epidermis (Fig. 2, G). They are numerous on the lobes but gradually become less frequent in the middle region of the calyx until at the base they are rare or absent.

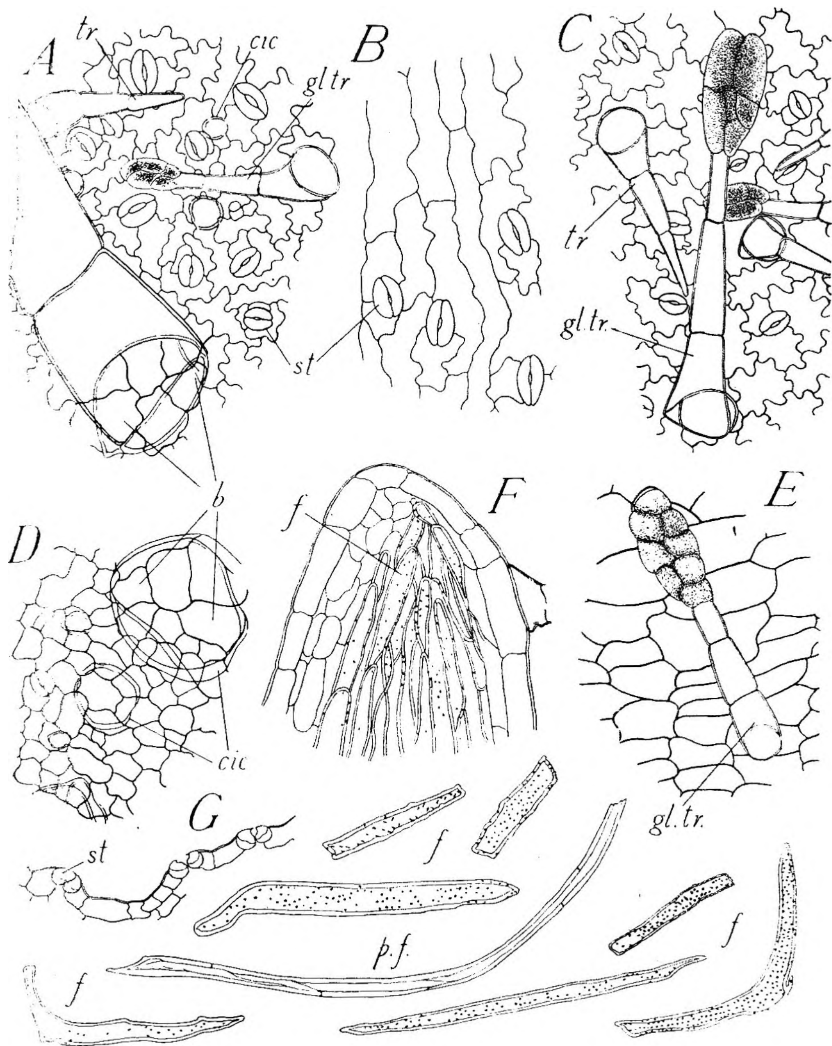

FIG. 2.—*Hyoscyamus niger* Linn., Calyx. A, Outer epidermis of a lobe. B, Outer epidermis over midrib near the tip of a lobe. C, Inner epidermis of a lobe. D, Outer epidermis at the base of the tube with the trichomes removed. E, Inner epidermis at the base of the tube. F, Longitudinal section through the tip showing the bundle of fibres strengthening the spine. G, Transverse section through the outer epidermis at the middle of a sepal showing details of the stomata. *b*, large epidermal cells under a trichome; *cic*, cicatrix; *f*, xylem fibres; *gl. tr*., glandular trichome; *p.f*., part of a pericyclic fibre, half in length; *st*, stoma; *tr*, covering trichome. All × 150.

Numerous covering and glandular *trichomes* occur on the entire epidermis, but are more crowded and longer towards the base of the calyx.

Inner or upper epidermis: The cells are similar to the corresponding cells of the outer epidermis (Fig. 2, C), except at the base where they are comparatively larger (Fig. 2, E), measuring about L and T = 9 to **40** to 140 μ and R = 7 to 12 μ. Numerous cruciferous stomata appear in all parts of the epidermis, although they are not so frequent at the base of the tube. *Trichomes,* similar to those on the lower epidermis, occur on the upper but are not so numerous. Very large trichomes similar to those on the outer surface of the base of the tube are absent, the basal portion being nearly glabrous.

Trichomes: Two types of trichomes occur on the calyx, glandular and covering. (a) *Glandular trichomes,* which are more numerous and resemble those of the foliage leaves, usually have a 1 to 4-celled uniseriate stalk and a large multicellular, ovoid or club-shaped glandular head containing yellowish-brown contents. The larger glandular trichomes occur chiefly on the lower part of the calyx and have a uniseriate stalk of up to 12 cells. The glandular trichomes frequently contain small crystals of calcium oxalate in the form of prisms, rosettes, single or double pyramids, the latter sometimes with truncated ends, micro-crystals of indeterminate shape and abundant minute globules of volatile oil. The oil appears as globules which stain yellowish-brown with iodine solution and orange-yellow with Scarlet R solution. The globules are soluble in alcohol and can be reprecipitated by addition of water; the tests indicating presence of volatile oil. The oil globules are present in dried specimens, but are absent from samples preserved in alcohol which has dissolved the oil. The majority of the glandular trichomes measure about 270 to **900** to 2160 μ in length, the larger ones being sometimes as long as 1 cm.; diameter at the base being 50 to 180 μ × 40 to 90 μ. The cicatrix left by a large glandular trichome appears as a prominent ring in surface view and may cover one much enlarged cell or 2 to 12 large epidermal cells, the latter being larger than the adjoining ones and having a less wavy outline (Figs. 2, A and D). (b) *Covering trichomes,* short, erect, uniseriate, 1 to 4-celled and tapering, are numerous over the entire calyx; occasional longer uniseriate trichomes have a stalk of up to 12 cells, the terminal cell of these long trichomes being either bluntly conical or cylindrical and rounded at the tip. They occur chiefly on the outer surface of the base of the tube. The covering trichomes are 105 to **175** to 228 μ long, the very long trichomes attaining a length of about 3·5 mm.; diameter at the base approximately 25 to 35 μ.

Mesophyll: This consists of 4 to 7 layers of thin-walled parenchyma irregularly arranged, the number of layers and the size of individual cells decreasing from apex to base, where the cells are also more closely packed, so that the calyx is thinner at the base than at the apex (Fig. 3). Calcium oxalate *crystals* are abundant in the mesophyll of the calyx. They occur as prisms or irregularly shaped crystals, either singly or in small groups and increase in density from apex to base. Idioblasts, containing a few large triangular shaped crystals together with a mass

of micro-crystals of various forms, triangular ones being more frequent, also occur in the tube and become more frequent towards the base. In the fruit, the calcium oxalate crystals increase in the calyx, appearing as clusters and micro-sphenoidal masses in the tube and as isolated square prisms or groups in the lobes. The increase in the number and dimen-

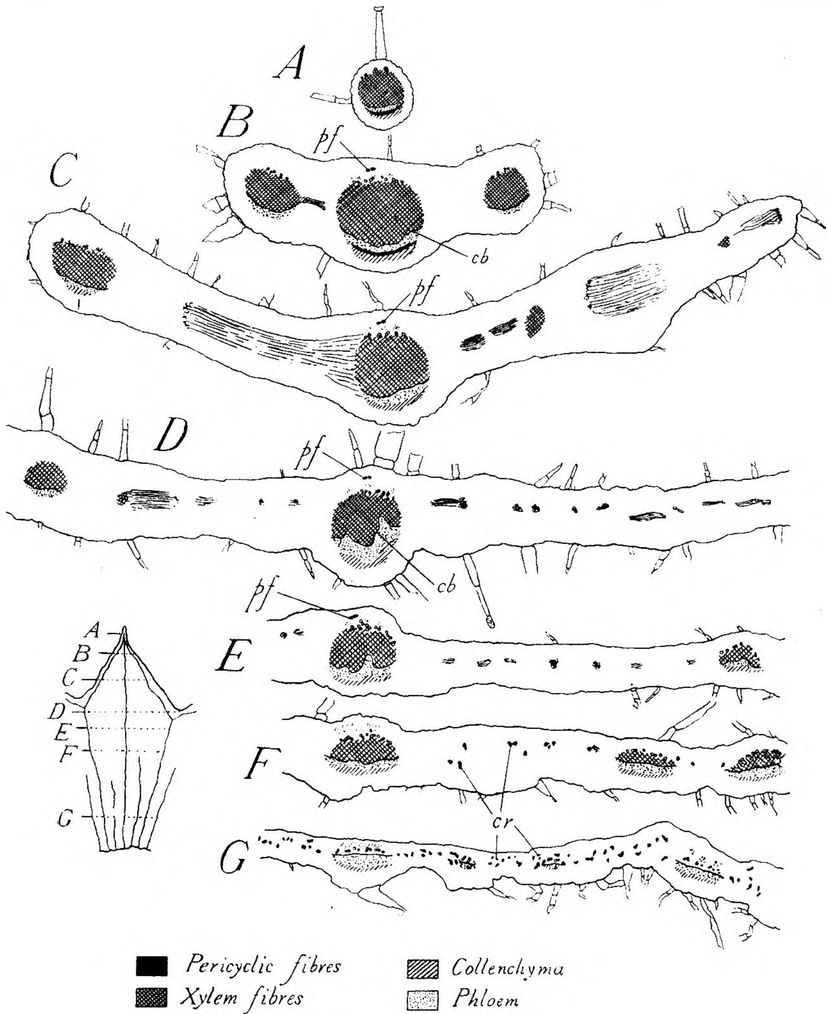

FIG. 3.—*Hyoscyamus niger* Linn., Calyx. Series of 7 transverse sections through a sepal showing diagrammatically the thinning of the lamina from apex to base, the distribution of tissues in the midrib and of the microsphenoidal crystal idioblasts at the base. The diagram in the corner at the bottom indicates the position on the sepal at which each section was cut. *cb*, cambium; *cr*, crystal-sand idioblast; *p.f.*, pericyclic fibre. All sections × 25.

sions of the calcium oxalate crystals as the calyx grows older agrees with the findings of Anselmino and Gilg[7] who examined foliage leaves of gradually increasing ages with a similar result as to the accumulation of calcium oxalate.

Venation: (Fig. 1, D). Each sepal has a well marked midrib which terminates in the spine of the lobe; between the midribs is a further well marked vein ending at the sinus between the lobes and joining with the prominent marginal vein, the latter running up to and strengthening the spine from either side. Between this prominent marginal vein and the actual margin of the calyx, there is a much more delicate marginal vein connected with the stronger one by numerous very slender branches. Beyond this more delicate marginal vein there are only a very few projecting vascular elements. As the two marginal veins approach the spine of the calyx lobe, they unite to form a single strong vascular strand. Branches also enter the cusps which are usually well developed in the fruit and contain strongly lignified elements (Fig. 1, F). Occasionally, in place of the single well-marked vein between the midribs, there occur two parallel veins; these represent the lateral veins of the sepals which, although normally fused, have escaped fusion[8] (Fig. 1, E). Several short, thinner veins arise at the base of the tube running vertically and parallel to the main veins and ending at different levels in the lower half of the tube. All the veins anastomose freely forming a dense network (Fig. 1, D).

In transverse sections (Fig. 3) the vascular strand of each midrib is small and oval in outline at the base of the calyx, gradually becoming larger and more circular in the lobes due to the gradual development of a mass of lignified xylem fibres with bluntly pointed ends. At the tip itself, the midrib is further enlarged by fusion with the two marginal veins from either side; the group of xylem fibres becomes enlarged and pericyclic fibres arise in the otherwise collenchymatous pericycle. The resulting cylinder of fibres forms the bulk of the spine and makes it remarkably stiff (Fig. 2, F). In the persistent calyx of the fruit, a development of lignified xylem fibres usually takes place in the vascular strands of the other larger veins also.

Histology of the Corolla

Outer or lower epidermis: The epidermal cells vary greatly from apex to base. On the lobes, the anticlinal walls have well-marked infoldings which are very characteristic (Fig. 5, Ao). Over the veins, the cells are elongated, do not have infolded anticlinal walls and are slightly papillose (Figs. 5, Av and 4, B). The infoldings gradually disappear towards the base, the cell walls becoming merely sinuous and finally straight; the cells at the base being sub-rectangular and comparatively thick walled (Figs. 5, Do, Eo and Fo). Each cell possesses a well-marked nucleus which is centrally placed on the inner wall, except at the base of the corolla, where it lies against one of the side walls. On the lobes of the petals, the epidermal cells measure approximately L and T = 25 to **40** to 63 μ and R = 11 to **14** to 25 μ; over the veins some of the cells may be as long as about 125 μ. Further down, near the lower part of the lobes, the cells are larger. At a height of about 1/3rd from the base of the petal, the cells measure approximately L = 90 to **100** to 163 μ, T = 15 to **30** to 40 μ and R = 13 to **20** to 35 μ. In the basal part of

the tube, the cells measure about, L = 30 to **63** to 90 μ, T = 15 to **25** to 38 μ and R = 40 to **45** to 55 μ. Occasionally, cruciferous *stomata,* slightly raised above the general surface of the epidermis, occur towards the base of the corolla, but are rather rare on the lobes. The stomata measure approximately 38μ long and 20μ broad. Short stalked glandular trichomes with a 1 to 4 celled uniseriate stalk and a small head

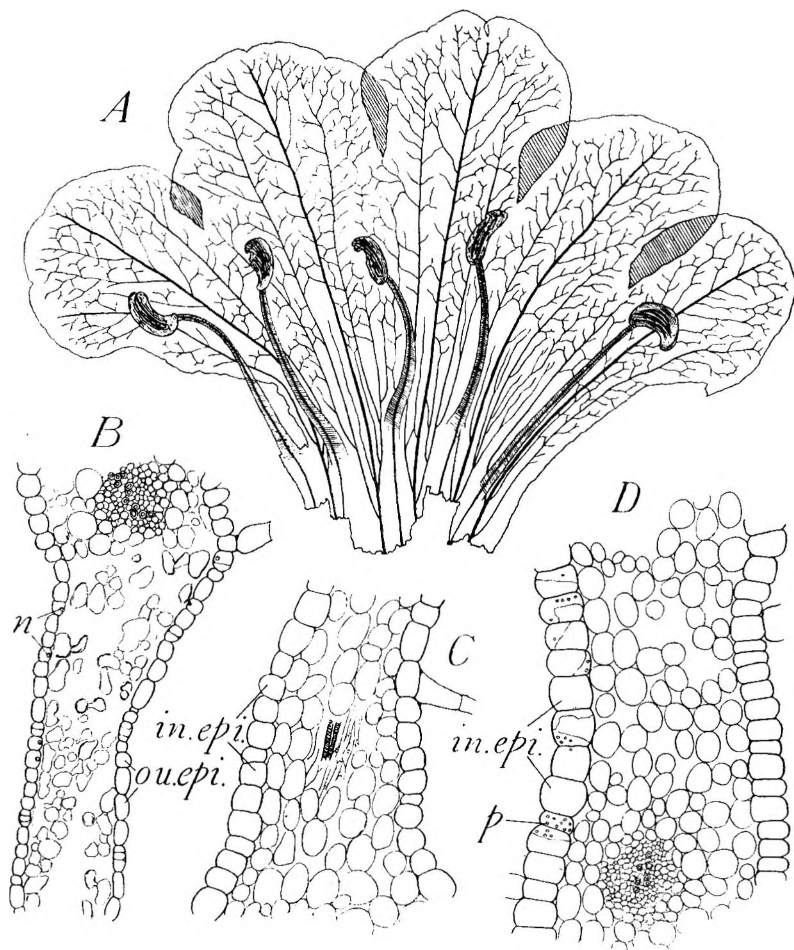

FIG. 4.—*Hyoscyamus niger* Linn., Corolla. A, Corolla spread out showing venation and stamens (× 3). B, Transverse section through the lobe of a petal. C, Transverse section through the middle of a petal. D, Transverse section through the tube at a position just above the actual base. *in. epi.*, inner epidermis; *n*, nuclei; *ou. epi.*, outer epidermis; *p*, pits on anticlinal walls of the inner epidermis. All × 100 except A.

of 1 to 4 cells or, more commonly, a large head of 10 to 30 cells filled with yellowish-brown contents are numerous, but much less so than on the calyx. The stalks of these glandular trichomes are longer towards the base of the tube. The trichomes often contain minute oil globules.

Scattered uniseriate covering trichomes, about 1 to **3** to 4 celled, also occur, but are comparatively rare. The glandular trichomes measure about 144 to **270** to 630 μ long, have a diameter of about 36 to **54** to

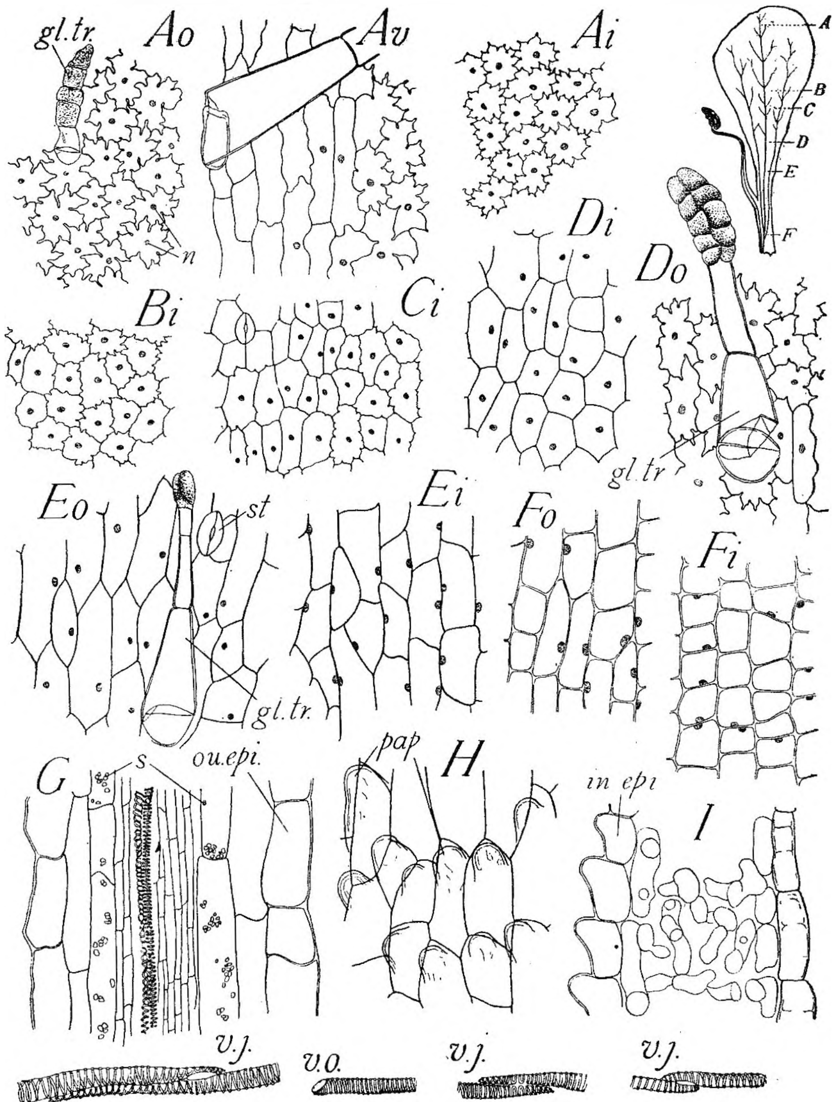

FIG. 5.—*Hyoscyamus niger* Linn., Corolla. *Ao,* outer epidermis at A (as marked on the diagram, top right); *Av,* outer epidermis at A over a vein; *Ai,* inner epidermis at A; *Bi,* inner epidermis at B; *Ci,* inner epidermis at C; *Di,* inner epidermis at D; *Do,* outer epidermis at D; *Eo,* outer epidermis at E; *Ei,* inner epidermis at E; *Fo,* outer epidermis at F; *Fi,* inner epidermis at F. G, longitudinal section through the basal part of the tube passing through a vein and showing starch grains. H, surface view of inner epidermis of the tube near position E showing papillæ; I, longitudinal section showing a sectional view of the papillose cells represented in H. *gl. tr.,* glandular trichome; *in. epi.,* inner epidermis; *n,* nucleus; *ou. epi.,* outer epidermis; *pap,* papilla; *s,* starch grain; *st,* stoma; *v.o.,* end of an isolated vessel from the corolla tube showing opening; *v.j.,* vessel junction from isolated xylem elements of the tube. All × 150.

90 μ at the base; the head measures 18 to **126** to 180 μ in length and 18 to **54** to 72 μ in diameter.

Inner or upper epidermis: On the lobes, the epidermal cells are similar to the corresponding ones on the outer epidermis (Figs. 5, Ai and Bi); over the veins, however, the infoldings disappear and the cells are slightly papillose. The cells on the lobes measure approximately L and T = 25 to **35** to 50 μ and R = 7 to **11** to 35 μ. On the lower part of the lobes, occasional cells measure as much as L and T = 75 μ and R = 40 μ. The cells on the tube gradually lose their infoldings (Figs. 5, Ci and Di), and at about the middle of the tube they are polygonal with only slightly curved anticlinal walls and often bear a small papilla arising from the end of the upper surface of the cell and directed towards the throat of the corolla (Figs. 5, H and I). The cells on the upper part of the tube measure L = 38 to **63** to 113 μ, T = 20 to **30** to 38 μ and R = 40 to 60 μ. Lower down, the cells are sub-rectangular, have comparatively thick lignified walls and bear pits on their anticlinal walls (Fig. 4, D). At the very base, the cells are more square in shape than rectangular, i.e., they decrease in length from the ones immediately preceding them, and measure L = 20 to **25** to 50 μ (Figs. 5, Ei and Fi). The epidermal cells on the lobes have a distinct centrally placed nucleus, whereas the rectangular ones at the base have their nuclei lying against the side walls. *Stomata* are rare or absent on the lobes but a few occur on the tube, being usually slightly depressed below the epidermal surface. A few *trichomes* are present on the lower part of the tube, but are very rare on the lobes.

Mesophyll: The mesophyll consists of about 3 to 10 layers of loosely arranged parenchymatous cells, the number of layers and the size of individual cells increases from apex to base, so that the petals are thinnest at the apex (Figs. 4, B, C, and D). Calcium oxalate *crystals* are rare in the lobes, but a number of isolated tetragonal prisms, truncated octahedra or irregular crystals are present in the tube, particularly in the parenchyma along the main veins. The parenchymatous cells adjacent to the main veins in the tube usually contain numerous small starch grains, either single or two-compound (Fig. 5, G). The grains do not polarise clearly, but they stain purple with solution of iodine.

Venation: 10 main veins arise from the base of the corolla tube; 5 enter the filaments, and each of the other alternating 5 veins enters a petal and gives rise to 2 main branches very near the base (Fig. 4, A). Thus each petal has 1 main vein and 2 main branches, all of which freely anastomose with each other, the finest veinlets running to within 3 to 15 cells of the edge of the lobes. The xylem elements of the veins of the petals consist of slender spiral vessels having circular or oval openings on the flat or tapering ends of the segments (Figs. 5, v.j. and v.o.).

Histology of the Stamens

Filament: The *epidermal cells* are polygonal in surface view, elongated in the direction of the axis of the filament and are covered with a moderately thick cuticle (Figs. 6, A and B). At the base, they merge in form and shape with those of the rectangular cells of the corolla at the

adnation. The cells measure approximately L = 53 to **105** to 140 μ, T = 10 to **18** to 25 μ and R = 21 to **35** to 70 μ. *Stomata* are absent. The *trichomes* are mainly glandular with comparatively small heads and uniseriate stalks which are very wide at the base and taper rapidly towards the middle of the stalk; dimensions, length about 140 to **350** to 560 μ and diameter at base = 40 to **60** to 150 μ; a few covering trichomes also occur (Figs. 6, *gl. tr.* and *tr.*). The trichomes are abundant at the base and become progressively smaller and less frequent towards the connective, in which region they are absent. The *cortex* consists of 5 to 6 rows of rounded, loosely arranged parenchyma containing numerous small scattered *crystals*, in the form of prisms or of indeterminate shape, being most abundant at the base and along the vascular strand which is centrally placed (Fig. 6, B).

Anther: The filament extends into a triangularly pyramidal *connective*, which has rectangular epidermal cells with a faintly striated cuticle and fairly numerous, rounded and usually open *stomata*. The cortical cells of the connective have numerous crystals, usually in the form of tetragonal prisms or of irregular shapes. The *lobes* are covered with an epidermis having a distinctly striated cuticle and consisting of isodiametric cells and cruciferous (anisocytic) *stomata* (Fig. 6, H); *trichomes* are absent. At the line of dehiscence, the outer epidermis in surface view takes the form of about two rows of small, thin-walled tabular cells measuring approximately L and T = 7 μ and R = 25 μ (Fig. 6, G). The epidermal cells gradually increase in size away from the stomium, becoming rounded polygonal in shape with prominent nuclei and on the lobes measure approximately L and T = 7 to **20** to **50** to 75 μ and R = 25 to 63 μ. The *fibrous layer* is several layers thick near the connective but gradually decreases in thickness to a single layer near the stomium where it is absent (Fig. 6, G). The walls of the pollen sacs at the stomium are held together only by the layer of small epidermal cells which form the line of dehiscence. The cells of the fibrous layer measure approximately L = 38 to **50** to 63 μ, T = 13 to **20** to 25 μ and R = 25 to 38 μ. They have a suberised band of thickening in the form of a spiral which appears in surface view as rods with thickened or beaded ends (Figs. 6, F and G). These spiral bands often fail to give a reaction for lignin when tested with phloroglucin and hydrochloric acid, though some fragments of the wall seen in surface view show a faint pink tinge, indicating partial lignification of the thickenings. Within the fibrous layer, remains of the tapetum are sometimes visible. The anthers dehisce at an early stage during the development of the flower.

The *pollen grains* are spherical; about 35 to **48** to 56 μ in diameter, with three equidistantly placed longitudinal germinal furrows, the ends of which taper towards the poles, but do not meet; in the centre of each furrow is situated a large pore about 10 to 18 μ in diameter; the exine bears numerous irregularly arranged minute rounded pits of slightly varying sizes[9,10] (Figs. 6, p_2 and p_3). In the immature grain, there are three inward folds along the position where the pores will develop (Fig. 6, p_1). The pollen grains contain oil globules which stain yellow

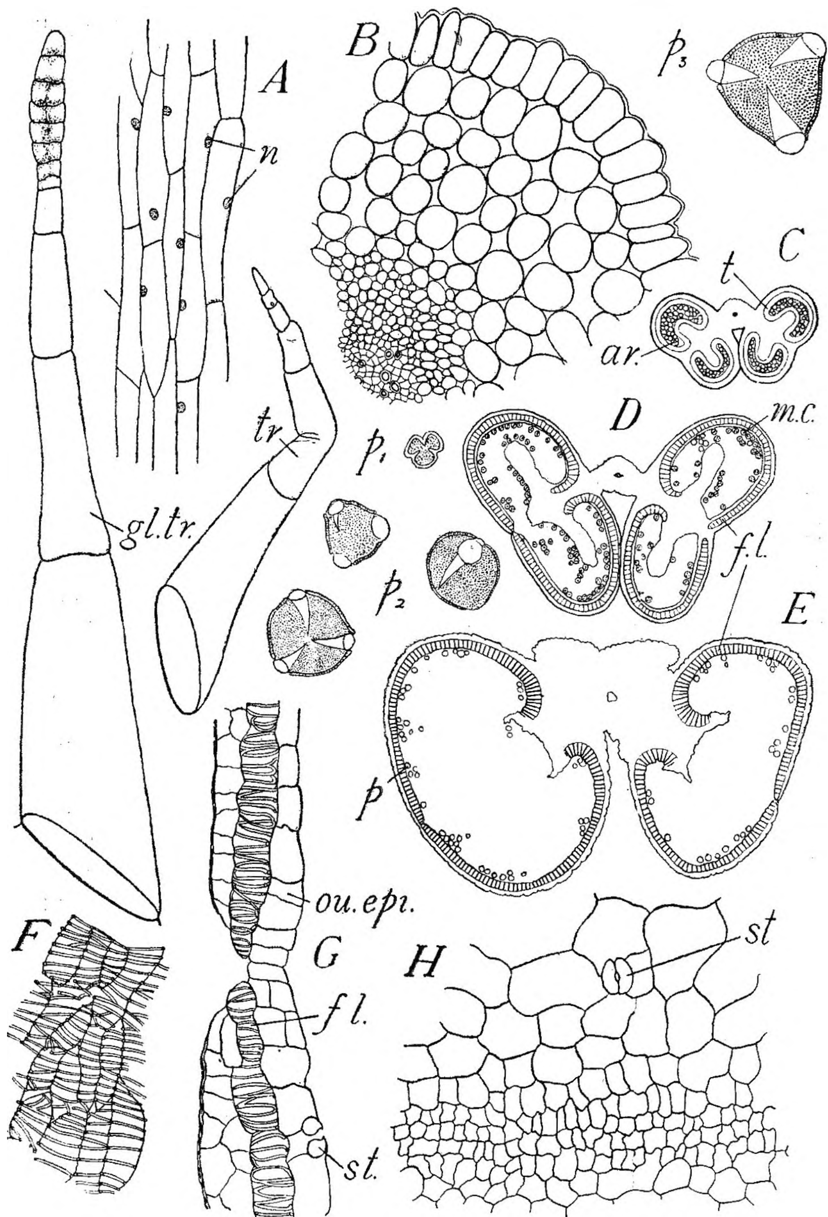

FIG. 6.—*Hyoscyamus niger*, Linn., Stamen. A, Epidermis from filament; B, Transverse section through filament; C, Transverse section through an anther showing the archesporium (×25); D, Transverse section through an anther showing mother cells containing immature pollen grains (×25); E, Transverse section through a mature anther containing mature pollen grains (×25); F, Fibrous layer of anther in surface view; G, Transverse section of anther-wall at line of dehiscence; H, surface view of epidermis over anther at the line of dehiscence. *ar.*, archesporium; *f.l.*, fibrous layer; *gl.tr.*, glandular trichome from base of filament; *m.c.*, pollen mother cell; *n*, nuclei; *ou.epi.*, outer epidermis; *p*, mature pollen grain; p_1, immature pollen grain; p_2, mature pollen grains; p_3, pollen grain further enlarged (×350); *st.*, stoma; t, tapetum; *tr*, covering trichome from filament. All ×200 unless otherwise specified.

in solution of Sudan III; they also contain numerous minute starch grains which stain purple in iodine solution after treatment with solution of chloral hydrate for about two minutes; their diameter then measures about 1 to 5 μ[11].

Histology of the Carpels

The *stigma* is rounded capitate, with a small oval funnel-shaped depression in the centre extended as a shallow groove reaching almost to the margin on either side and indicating its origin from two carpels (Figs. 7 A, B and E). Its surface is covered with papillæ. The papillæ contain oil globules which often float out in the mountant and stain orange yellow in solution of Sudan III. The papillose cells measure about 15 to **120** to 175 μ in length and about 18 to **28** to 36 μ in diameter at the base. In full-blown flowers, pollen grains, often with a growing pollen-tube, are adherent to the surface of the stigma. Numerous small crystals usually occur in the stigma.

The *style* is about 8 to 20 mm. long and 0·2 to 0·7 mm. in diameter. The epidermal cells in the lower part of the style, which is gradually thicker towards the base, are tabular, elongated along the axis and similar to those of the filament. The epidermal cells measure about L = 35 to **88** to 140 μ, T = 11 to **14** to 25 μ and R = 11 to 18 μ; at the base of the style, the dimensions correspond to the lower limits. In this region, *stomata* are absent and 1 to 5 celled conical covering *trichomes*, with a rather rounded tip, occur. The trichomes measure about 170 to 300 μ in length and 18 to **28** to 50 μ in basal diameter. The epidermal cells on the upper part of the style have a dome-shaped outer wall (Fig. 7, C). *Trichomes* are very rare in this region, but *stomata* occur frequently and are usually raised well above the general surface of the epidermis with a large oval opening between the guard cells; they do not show any special arrangement of the subsidiary cells. Calcium oxalate *crystals* occur occasionally as isolated prisms, rosettes or clusters; starch grains are abundant in the mesophyll of the style.

In transverse section, the style shows a single row of epidermal cells covered with a finely striated cuticle; at the centre, there is an oval core of very loosely arranged small rounded cells about 3 to **6** to 9 μ in diameter, sometimes with a central irregular lacuna; on either side of the core is a small vascular bundle, the remaining tissue consists of fairly large-celled uniform parenchyma cells about 6 to **18** to 30 μ in diameter, with numerous intercellular spaces, forming a surrounding layer about 12 cells wide (Fig. 7, D).

The Ovary: The *outer epidermis* (Figs. H and I) consists of tabular cells with almost straight anticlinal walls; they vary somewhat in size, the dimensions being, L and T = 5 to **15** to 30 μ and R = 11 to 18 μ. *Stomata* are present and are more numerous on the lower part where they are much larger than the adjoining cells and the pores are open. The subsidiary cells do not show typical cruciferous arrangement. On occasional ovaries, a very few short covering *trichomes*, 1 to 3 cells long, occur on the outer epidermis of the upper part. The *inner epidermis*

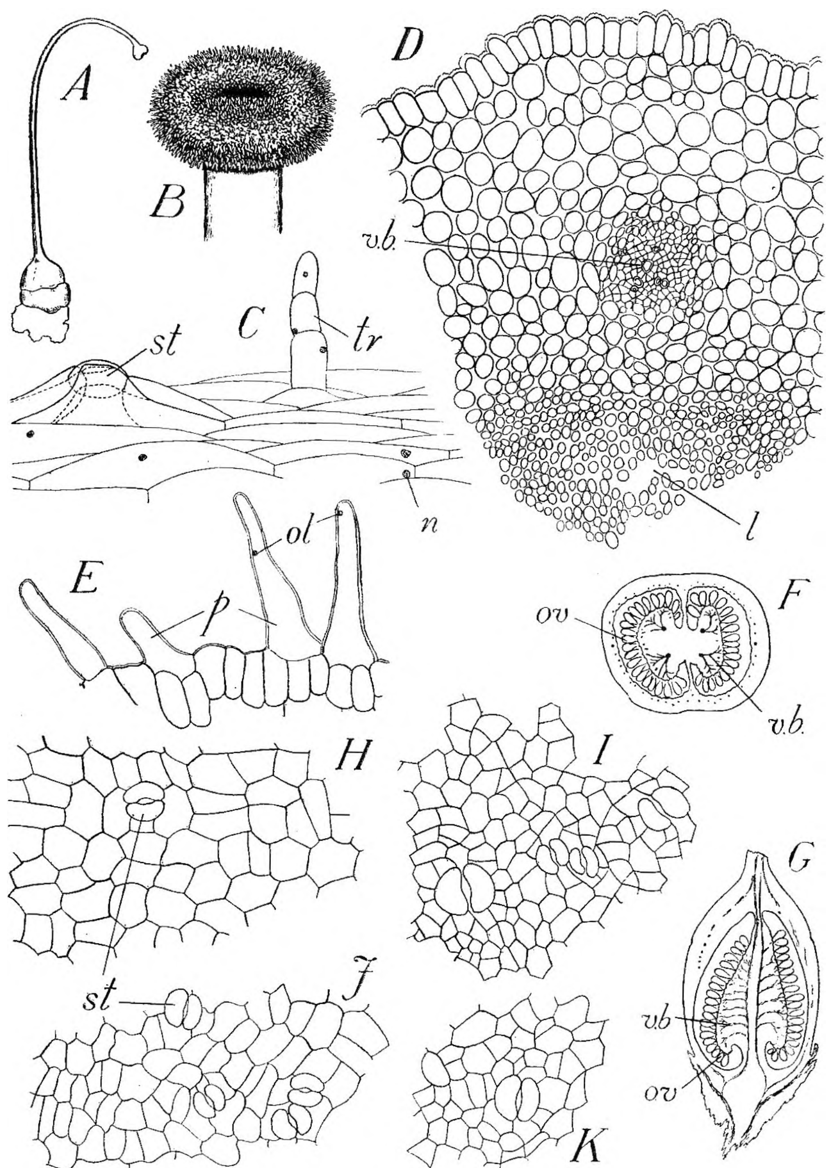

FIG. 7.—*Hyoscyamus niger*, Linn., Carpels. A, Entire gynœcium ×2. B, Stigma ×12. C, Epidermis of style, tangential surface view. D, Transverse section of style. E, Section through the epidermis of a stigma showing papillose cells. F and G, Diagrammatic drawings respectively, of transverse and longitudinal sections of the ovary ×12. H, Outer epidermis over apical part of the ovary. I, Outer epidermis near base of the ovary. J, Inner epidermis of apical part of the ovary. K, Inner epidermis at the base of the ovary. *l*, lacuna; *n*, nucleus; *ol*, oil globules; *ov*, ovule; *p*, papilla; *st*, stoma; *tr*, covering trichome; *v.b.*, vascular bundle. All drawings ×200 unless otherwise specified.

consists of cells similar to those of the outer epidermis, but *stomata* are more frequent (Figs. 7, J and K).

The *mesophyll* consists of closely arranged polyhedral parenchymatous cells with small intercellular spaces, many of the cells being filled with minute starch grains particularly in the upper part of the ovary; the mesophyll is traversed by slender vascular strands. *Crystals* often occur in the cells of the mesophyll, as single isolated indeterminately shaped or as groups of several large crystals.

The epidermal cells of the *septum* and the *placenta* vary between sub-rectangular to isodiametric and are often similar to those of the epidermis of the ovary wall. Stomata are rarely present. The ground tissue of the central part of the septum and of the placenta consists of loosely arranged isodiametric parenchymatous cells. Small microcrystals of odd shapes are abundant in the cells of the placenta and also occur frequently in the septum. As the ovary matures, the crystals acquire a definite structural form, being usually present as double pyramids, sometimes with truncated ends, cubes, right angle prisms or hemihedral crystals.

Ovules: Very young ovules consist of undifferentiated parenchymatous cells.

Powdered Flowers

Henbane flowers, dried and powdered to No. 90 mesh, result in a greenish-brown powder with a typical unpleasant odour. The powder was examined after mounting it in the following: dilute glycerin, iodine solution, solution of chloral hydrate, lactophenol and in phloroglucin and hydrochloric acid. The structures which are of greatest use in the identification of the powdered flowers are arranged in order of importance as follows:

1. Pollen grains, mature grains about 40 to 50 μ in diameter having 3 pores and 3 furrows; the exine with very numerous irregularly scattered rounded pits (Figs. 8, I_1 and I_2).

2. Fragments of the anther wall, which impart a pink tinge to solution of chloral hydrate owing to the presence of anthocyanin, and include the characteristic fibrous layer which sometimes gives a slight reaction for lignification (Figs. 8, C and D).

3. Broken pieces of trichomes, especially the large multicellular club-shaped or ovoid glandular heads with granular and yellowish contents (Figs. 8, F, G, L and P).

4. Fragments of the upper and lower epidermises of the corolla lobes with characteristic infoldings of the anticlinal walls; the fragments yielding a transient pink colour in solution of chloral hydrate (Fig. 8, A).

5. Fragments of lignified vascular strands, appearing as slender spiral elements, sometimes associated with thick walled, pitted xylem fibres from the calyx (Figs. 8, K and M).

6. Lignified rectangular cells of the lower part of the corolla tube, sometimes bearing rounded, simple pits on their anticlinal walls (Figs. 8, B and E).

7. Fragments of the epidermises of the ovary composed of straight thin-walled polygonal cells; occasional stomata are present. (Fig. 8, J).

8. Fragments of the calyx lobes with wavy walled epidermal cells and cruciferous stomata. Small uniseriate covering trichomes are present on the epidermis and occasional idioblasts containing prisms or micro-sphenoidal crystals occur in the mesophyll. Certain fragments from the base of the outer epidermis of the calyx have large cicatrices left by the larger trichomes (Figs. 8, G, H, K, cr and N).

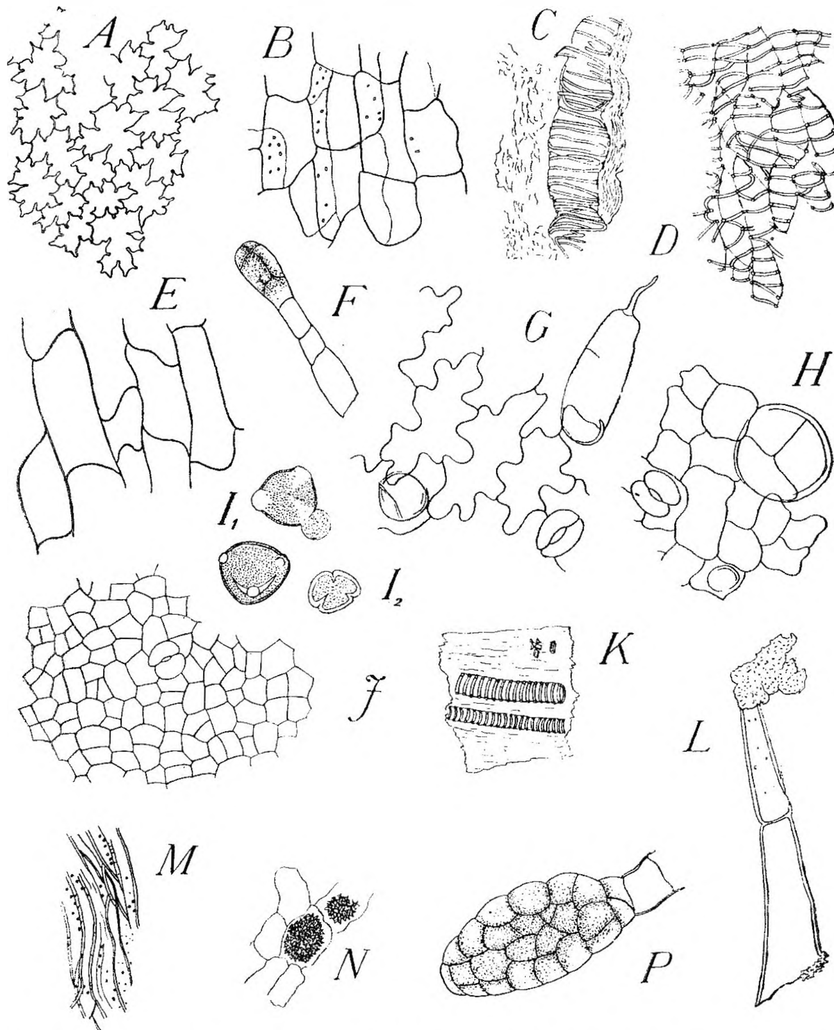

FIG. 8.—*H. niger*, Linn., Powdered Flowers. A, Epidermis of corolla lobe. B and E, Epidermis near the base of the corolla tube. C, Anther wall in sectional view. D, Fibrous layer in surface view. F, Glandular trichome. G, Epidermis from calyx lobe. H, Outer epidermis from base of calyx tube. I_1, Mature pollen grains. I_2, Immature pollen grain. J, Epidermis of ovary wall. K, Spiral vessels of the calyx with a few crystals. L, Broken glandular trichome. M, Group of lignified fibres from a vein of the calyx. N, Micro-sphenoidal crystal sand. P, Glandular head of a large trichome from the calyx. All ×200.

SUMMARY

A. A brief review of the published description of the gross morphology of the flower of *Hyoscyamus niger* Linn. has been made and a somewhat more detailed new account of the macroscopical characters is given. This is followed by a detailed illustrated description of the microscopical characters, the following being of especial diagnostic value:

1. *Calyx:* Epidermal cells varying from wavy-walled on the lobes to straight-walled at the base; cruciferous stomata numerous; glandular and covering trichomes long and numerous; abundant microsphenoidal crystals present as single crystals or as sandy masses; xylem fibres present in the midrib of the lobes.

2. *Corolla:* Epidermal cell walls with well-marked infoldings on the lobes, straight at the base; trichomes, mostly glandular, frequent on outer epidermis; stomata, rare, present on the tube only; small starch grains in cells adjacent to the spiral vessels of the xylem in the tube.

3. *Stamens: Filament,* stomata absent; trichomes mostly glandular, crowded towards the base but scanty elsewhere; crystals in the ground tissue. *Anther,* stomata on epidermis, fibrous layer slightly lignified, crystals in connective. *Pollen grains* spherical, with three germinal furrows and three pores; exine marked with numerous irregularly scattered pits; contain oil globules and starch grains; mature pollen grains measure about 40 to 50 μ in diameter.

4. *Carpels: Stigma* capitate with a funnel-shaped depression in the centre, covered with papillæ; *style,* epidermis bears stomata and occasional trichomes; crystals and starch grains in ground tissue. *Ovary,* bilocular, stomata on inner and outer epidermises of the wall; crystals present; *ovules* numerous.

5. *Powder:* The powder of the flowers of *Hyoscyamus niger* may be identified by the characters of the pollen grains, the fibrous layer of the anther walls, the large multicellular, ovoid glandular heads of the trichomes, the cells of the corolla lobes and the xylem fibres from the main veins of the calyx.

B. The chief histological features which distinguish the flower of *Hyoscyamus niger* Linn. from that of *Atropa belladonna* Linn.[12] are:

1. *Pollen:* The pollen grains of both flowers are very similar in size and form. The pores on the exine, however, are irregularly scattered in the pollen of *H. niger,* but are arranged in rows radiating from the poles in case of *A. belladonna*[9,10].

2. *Trichomes:* Covering trichomes are absent from the calyx of *A. belladonna,* but upon that of *H. niger* they occur in considerable numbers associated with the typical glandular trichomes. The glandular trichomes of *H. niger* are clearly distinguished by their large ovoid multi-cellular glandular heads, whereas in *A. belladonna* the glandular heads are usually small, spherical and unicellular.

3. The cuticle of the calyx in *A. belladonna* is finely striated; whereas that of *H. niger* is smooth.

4. The main veins of the calyx in *H. niger* have numerous lignified pitted xylem fibres, while those of *A. belladonna* are devoid of fibres.

5. The anticlinal walls of the epidermal cells of the lobes of the corolla in *H. niger* have typical deep infoldings; the corresponding cells of the outer epidermis of the corolla of *A. belladonna* have very sinuous anticlinal walls, and those of the inner epidermis have nearly straight walls and well-marked small papillæ.

C. The flowers of *Datura stramonium* are readily distinguished from those of *H. niger* by one well-known character, viz., the size and markings of the pollen grains[9,10], which are about 56 to 80 μ in diameter and have a coarsely granular exine in case of *D. stramonium*. Further work on the flower of *D. stramonium* is in progress.

ACKNOWLEDGEMENTS

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REFERENCES

1. Bentley and Trimen, "*Medicinal Plants,*" 1880, **3,** 194.
2. Moll and Janssonius, "*Botanical Pen Portraits,*" 1923, 325.
3. Berg, "*Anatomischer Atlas zur Pharmazeutischen Waarenkunde,*" 1865, 93, plate XXXXVII.
4. Tschirch und Oesterle," "*Anatomischer Atlas der Pharmakognosie und Nahrungsmittelkunde,*" 1900, 167, plate 39.
5. Sachs, "*Text book of Botany,*" 2nd ed., 1882, 598.
6. Metcalfe and Chalk, "*Anatomy of the Dicotyledons,*" Oxford University Press, 1950, **1,** xv.
7. Anselmino und Gilg, "*Die Bilsenkrautblätter des Handels,*" *Arch. Pharm.*, 1913, **251,** 367.
8. Eames and MacDaniels. "*An Introduction to Plant Anatomy,*" 1947, 347.
9. Zander, "*Beiträge zur Herkunftsbestimmung bei Honig,*" "*Pollengestaltung und Herkunftsbestimmung bei Blütenhonig,*" Berlin. 1935, 279.
10. Wallis, "*The Study of Pollen,*" *Pharm. J.*, 1947, **158,** 8, 29.
11. Lang, "*Investigations of the Pollen of the Malvaceæ, with special reference to the inclusions,*" J.R. micr. Soc., 1937, **57,** 75.
12. Wallis and Butterfield, "*The flower of Atropa belladonna Linn.,*" *Quart. J. Pharm. Pharmacol.*, 1939, **12,** 511.

ANALYSIS OF THE ACCESSORY FACTORS IN THE CAUSATION OF DERMAL REACTIONS TO INSULIN

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Local reactions in the skin were observed soon after the introduction of insulin for the treatment of diabetes mellitus, and one of the first papers to record this phenomenon was by Joslin[1] and his co-workers in 1922. They stated that induration at the site of injections of insulin was frequent and described four cases of urticarial wheals with pruritus. One patient developed a small crusted ulcer without evidence of infection, and it was suggested that this might possibly be due to a burn from tricresol, the preservative used.

Subsequently many authors[2,3,4,5] have described the local sensitisation phenomenon to insulin therapy. In a comprehensive article published in 1932, Allan and Scherer[6] considered that the local reactions at the site of injection might be due to chemical irritation such as:—1. High concentrations of salts used in early preparations of insulin. 2. Excessive amounts of tricresol used as a preservative. 3. Acidity of early preparations. 4. Injection of denatured alcohol used for cleaning skin and storing syringe. On the other hand, these local reactions might be due to hypersensitivity to insulin and could be ascribed to:—5. Pancreatic protein of the animal from which the insulin was obtained. 6. Insulin protein itself.

When the first diabetic patients were treated with insulin and complained of stinging and local reactions at the site of injections, Banting *et al.*[3] thought that the high salt content of the pancreatic extracts was the cause. The salt content was reduced in subsequent preparations, but certain samples of this insulin continued to give rise to local skin sensitivity. In 1923, Banting, Campbell and Fletcher[7] used a "practically protein free" insulin preparation which gave rise to urticarial eruption in only one or two sensitive patients under their care. By modern standards the early preparations were extremely crude, and in 1925 Campbell and Mcleod[8] considered that local reactions were steadily diminishing in number with the increasing purity of the insulin.

Wilder *et al.*[2] also described the local effects of insulin therapy and recorded a few cases where necrosis and sloughing of the skin had occurred. This reaction was attributed to the preservative—tricresol—contained in the preparation. It is rare to observe necrosis of the skin following injections at the present time, and the local reactions described by Joslin *et al.*[1] and Wilder *et al.*[2] may have differed fundamentally from those encountered to-day. More recently Leavitt and Gastineau[9] stated that preservative in the insulin solution may cause local reactions, but they cited no patient with necrosis of the skin. Although many authors have stated that preservative in the insulin

solution appears to be the irritating factor, no carefully controlled experiment has been published demonstrating this effect.

The distinctly acid reaction of insulin solution has been implicated as the cause of local erythema. In 1924, Stillwell[10] described a method of neutralising the acidity with 6 per cent. solution of sodium bicarbonate and 3 per cent. of tricresol. By injecting this neutralising solution with the insulin the author claimed that local reactions in a previously sensitive patient were abolished. More recently Page and Bauman[11] in a detailed investigation used an acid control solution prepared from potassium acid phosphate which had *p*H 3·5, similar to that of globin insulin. They tested a series of diabetic patients with this solution and only obtained 1·2 to 3·0 per cent. of positive reactions.

It has been suggested that injection of denatured alcohol used in cleaning the skin or storing the syringe may cause erythema. Allan and Scherer[6] reported that skin irritation in one of their patients was due to hypersensitiveness towards the formalin contained in the alcohol. Storage and sterilisation of the syringe and needles as well as injection technique were carefully checked in all patients of the investigation. In none could the injection of denatured alcohol be implicated.

Many workers have investigated the protein impurities in insulin preparations. Campbell *et al.*[12] used crystalline insulin, which had a reduced animal pancreatic protein content, and stated that the induration of the subcutaneous tissues and area of reddening of the skin surrounding the site of injection was less than with commercial insulin. This work has been confirmed by many workers[13,14,15,16]. During 1949, Paley[17] investigated the effect of insulin recrystallised six times. A group of diabetic patients reacting locally to various brands of insulin were tested intracutaneously with insulin from their current vial and also with the recrystallised insulin. A striking reduction in the mean area of reaction was seen with the purified insulin.

Whilst the investigation left no doubt that the main factor causing local sensitisation was some substance closely associated with insulin itself, it was suggested that there may be accessory factors such as the *p*H of the solution and also the retarding substance, salmine sulphate. The present investigations were devised to ascertain the effect of preservative, cresol B.P., *o*-cresol and other constituents of commercial insulins on the local sensitisation phenomenon.

Experimental Method and Results

Twelve diabetic patients were selected because they were showing erythematous reactions to injections of all brands of insulin.

Each patient received intra-cutaneous injections of various test solutions on the flexor surface of the forearm. In the first experiment three solutions were used:—

A. Sterile water adjusted to *p*H 3·0 to 3·2.

B. Sterile water adjusted to *p*H 3·0 to 3·2 with 0·3 per cent. of cresol B.P.

C. Injection of commercial soluble insulin (made from crystalline material) *p*H of the solution was 3·0 to 3·2 and it contained 0·3 per cent. of cresol B.P.

The test dose consisted of 0·02 ml. of each solution, and the reactions were read after 15 minutes interval and graphed.

Using the technique of intracutaneous testing it was found that these sensitive diabetic patients reacted to buffer solution alone. Table I shows the mean area of reactions for these patients to this solution to be 317·5 sq.mm. This effect, however, is reduced by the addition of 0·3 per cent. of cresol B.P. The difference between the mean areas is markedly significant statistically when " Student's t " test is applied to the data.

TABLE I

INTRACUTANEOUS REACTIONS TO TEST SOLUTIONS

SOLUTION	REACTION MEAN AREA sq. mm.	DIFFERENCE	" t "
A. Sterile Water :— *p*H. 3·0 to 3·2	317·5	245·4	6·5 t=2·797 P=0·01
B. Sterile Water :— *p*H. 3·0 to 3·2 ... ... +0·3 per cent. of cresol	72·1		
C. INSULIN :— *p*H. 3·0 to 3·2 ... ... +0·3 per cent. of cresol	456·9	384·8	3·368 t=2·797 P=0·01

A significant increase in mean area of reaction is observed when solution " C " (commercial insulin) is compared with solution " B " (sterile water, *p*H 3·0 to 3·2 + 0·3 per cent. of cresol B.P.). When insulin is compared with solution " A " (sterile water *p*H 3·0 to 3·2) an increase in mean area is observed but this is not significant.

From the data presented in Table I, it would appear that the reduction in area of reaction caused by the addition of cresol B.P. to sterile water, *p*H 3·0 to 3·2 is nullified when insulin is added. Comparison of solutions " B " and " C " suggest that the increase in area of reaction is due to some substance introduced by the addition of insulin. The minimal response to solution " B " may be explained by the local anæsthetic action of cresol. Burfoot[18] in a personal communication stated that:—" Injections of insulin in America usually contain phenol as a preservative, although *o*-cresol is used by at least one firm. In Great Britain cresol B.P. or *o*-cresol is used. The reason for the use of *o*-cresol is probably due to it being a pure chemical substance whereas cresol B.P. is a variable mixture of the ortho, para and meta isomers." Although cresol B.P. does not appear to exert any action in the genesis of local insulin sensitisation, the following experiment was devised in order to determine any possible difference between the action of these two preservatives.

Two test solutions were prepared containing the same batch of insulin and identical with respect to *p*H, differing only in so far as one contained 0·3 per cent. of cresol B.P. and the other 0·3 per cent. of pure *o*-cresol as preservative.

A group of 15 diabetic patients exhibiting dermal reactions to various

brands of insulin were then tested intracutaneously using 0·02 ml. of each solution. Table II shows that insulin containing *o*-cresol gave a larger mean area of reaction than the same insulin containing cresol B.P. The difference is not statistically significant.

TABLE II

COMPARISON OF THE EFFECT OF CRESOL B.P. AND *o*-CRESOL

SOLUTION	MEAN AREA OF REACTION sq. mm.	DIFFERENCE	"t"
INSULIN + CRESOL (Solution C)	574	186	1·249
INSULIN + *o*-CRESOL	750		t = 1·055 when P = 0·3

In 1936, Hagedorn *et al.*[19] combined insulin with a basic protein substance (protamine-salmine) obtained from the sperm of rainbow trout. By adjusting the hydrogen ion concentration of this solution to that of tissue fluids they precipitated the protamine insulin. The preparation was used successfully in the treatment of a number of diabetic patients. Subsequently Root[20] and his co-workers confirmed that protamine zinc insulin exerted a prolonged hypoglycæmic effect. During the early days of treatment with the protamine insulins cutaneous reactions were said to be absent. Typical local sensitivity to injections of protamine insulin, however, was reported by Kern and Langer[21]. Fowler *et al.*[22] had presented a single report of a patient showing sensitivity to this type of insulin as early as 1937. The former investigators reported no positive reaction in any subject, diabetic or control when tested intracutaneously with a simple solution of protamine containing 0·1 mg. of nitrogen per ml. Their animal investigations also revealed an inability to sensitise guinea-pigs to protamine. Yet, Joslin[23] considers that local responses are more common since the introduction of protamine zinc insulin. In view of conflicting evidence implicating protamine as a cause of local skin reactions, further investigation was undertaken. Three test solutions were used:—1. A phosphate buffer solution at *p*H 7·0 to 7·2. 2. A 0·052 per cent. sterile solution of salmine sulphate adjusted to *p*H 3·0 to 3·2 which prevents precipitation. 3. Ordinary commercial zinc protamine insulin containing insulin from the same batch as solution C, the *p*H was 7·0 to 7·2 and salmine was identical with test solution "2."

Intracutaneous tests of these solutions were performed on the 12 sensitive patients at the same time as the injections of solutions "A," "B" and "C." The mean area of reaction to phosphate buffer solution at *p*H 7·0 to 7·2 was small and measured 122·6 sq. mm. This result was not unexpected since the *p*H of body tissues is of the same order. The 0·052 per cent. sterile solution of salmine sulphate was prepared at *p*H 3·0 to 3·2 in order to keep it in solution and thus prevent inconsistent injections of a suspension. If the mean area of reaction to this solution is compared with that obtained from solution "A" (sterile water adjusted to *p*H 3·0 to 3·2) they are found to be almost identical (Table III).

TABLE III

EFFECT OF SALMINE SULPHATE

SOLUTION	MEAN AREA OF REACTION sq. mm.	DIFFERENCE
SOLUTION " A " (Water *p*H 3·0 to 3·2)	317·5	5·7
SOLUTION " 2 " :— (Water *p*H 3·0 to 3·2 +salmine sulphate)	323·2	

The findings presented here confirm Kern and Langer's[21] results that salmine does not cause skin reactions. Further indirect evidence is supplied by the mean area of reaction to intracutaneous test injections of commercial zinc protamine insulin (solution " 3 "). This was 430 sq. mm. and corresponds closely to the mean area of reaction obtained from test injections of commercial soluble insulin on the same series of patients, *viz.*, 456·9 sq. mm. (Table I).

In a previous communication Paley[17] demonstrated that the mean area of reaction to an intracutaneous injection of insulin recrystallised 6 times was 150·6 sq. mm. When this was compared with the mean reaction area obtained with test injections of different brands of insulin a significant reduction in area was observed.

The insulin recrystallised 6 times came from the same parent batch of insulin as solution " C " used in the present investigation. Concurrent tests were performed with solution " C " in the former experiment and a comparison, therefore, can be made with the highly purified insulin (Table IV).

TABLE IV

RESULT OF PURIFYING INSULIN

SOLUTION	MEAN AREA OF REACTION sq. mm.	DIFFERENCE
INSULIN :— (Solution C) 34 cases	388·1	237·5±52·9
INSULIN :— Recrystallised 6 times 34 cases	150·6	

It will be seen that there is a strikingly significant reduction in area of reaction to commercial soluble insulin when its insulin is recrystallised 6 times. This finding indicates that some " reacting factor " is removed during purification.

CONCLUSIONS

Intracutaneous testing in a large group of sensitive diabetic patients revealed some who gave reactions to substances other than insulin. Occasionally patients who did not react intracutaneously to the test insulin reacted to other constituents of their routine therapeutic insulin

solutions. Substances which gave rise most frequently to these reactions were sterile water, *p*H 3·0 to 3·2 and 0·052 per cent. salmine sulphate solution at same *p*H.

While aforementioned factors may operate in isolated cases, they did not appear to exert a significant effect on a series of sensitive patients. Cresol B.P. did not produce a reaction when injected intracutaneously in the strength supplied in commercial insulin, and no obvious advantage would appear to be gained by the use of *o*-cresol. Salmine sulphate when injected intracutaneously appears to be inert and cause no reaction. Comparison between commercial zinc protamine insulin and soluble insulin is important. No significant difference was shown between the mean areas for these two solutions when injected intracutaneously. Because of the widely differing composition of soluble insulin and zinc protamine insulin solutions, a direct comparison is not valid when investigating the mechanism of dermal reactions to insulin. Since preservative, salmine sulphate and *p*H do not appear to exert a dramatic influence on the skin, a similar mean area of reactions to both these " insulins " strongly supports the hypothesis that their common factor (insulin or closely associated substances) is the causal agent in the production of dermal reactions during insulin therapy. Stronger support for this hypothesis is provided by the marked reduction in reaction observed with highly purified insulin.

Summary

1. A brief historical review is given of possible factors involved in the production of local cutaneous reactions to insulin.

2. Neither the *p*H of the solution when given with cresol B.P. nor salmine sulphate appears to exert any irritant effect on the skin.

3. No statistically significant difference is observed in the area of reaction when pure *o*-cresol is substituted for cresol B.P.

4. It has been shown that these accessory factors cause little or no irritation of the skin, and the main factor in local sensitisation is some substance closely associated with insulin.

I wish to thank Messrs. Boots Pure Drug Co. for kindly supplying the test substances and Mr. P. Hey, Department of Pharmacology, University of Leeds, for preparing the solutions, also Professor R. E. Tunbridge for his helpful criticisms and advice.

References

1. Joslin, Gray and Root, *J. Metab. Res.*, 1922, **2**, 651.
2. Wilder, Boothby, Barborka, Kitchen and Adams, *J. Metab. Res.*, 1922, **2**, 701.
3. Banting, Campbell and Fletcher, *J. Metab. Res.*, 1922, **2**, 547.
4. Lawrence, *Lancet*, 1925, **1**, 1125.
5. Strauss, *Klin. Wchsch.*, 1925, **1**, 491.
6. Allan and Scherer, *Endocrinology*, 1932, **16**, 417.
7. Banting, Campbell and Fletcher, *Brit. med. J.*, 1923, **1** 8.

8. Campbell and Macleod. *Insulin*. The Williams and Wilkins Co., Baltimore, 1925, 77.
9. Leavitt and Gastineau, *Arch. intern. Med.*, 1947, **80**, 271.
10. Stillwell, *Brit. med. J.*, 1924, **2**, 465.
11. Page and Bauman, *J. Amer. med. Ass.*, 1944, **124**, 704.
12. Campbell, Gardiner and Scott, *J. clin. Invest.*, 1930, **9**, 28.
13. Baker, *Arch. intern. Med.*, 1936, **58**, 373.
14. Altshuler, *Ann. intern. Med.*, 1937, **11**, 901.
15. Ulrich, Hooker and Smith, *New Engl. J. Med.*, 1939, **221**, 522.
16. Jorpes, *Arch. intern. Med.*, 1949, **83**, 363.
17. Paley, *Lancet*, 1949, **256**, 1216.
18. Burfoot, *Personal communication*, 1949.
19. Hagedorn, Jensen, Krarup and Woodstrup, *J. Amer. med. Ass.*, 1936, **106**, 177.
20. Root, White, Marble and Stotz, *ibid.*, 1936, **106**, 180.
21. Kern and Langer, *ibid.*, 1939, **113**, 198.
22. Fowler, Bensley and Rabinowitch, *Canad. med. Ass. J.*, 1937, **36**, 561.
23. Joslin, *Treatment of Diabetes Mellitus*, Henry Kimpton, London, 1946, 465.

STUDIES OF RICINOLEIC ACID AND A TURBIDIMETRIC METHOD OF EVALUATING THE BACTERICIDAL ACTION OF SOLUTIONS OF PHENOLS IN POTASSIUM RICINOLEATE

PART II. PRELIMINARY INVESTIGATIONS IN THE USE OF THE NEPHELOMETER

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THERE are many methods available for the evaluation of bactericidal action, and these may be classified as follows. (1) End point methods in which tests for sterility are performed on samples taken after a fixed time or at predetermined time intervals from a test solution of the bactericide which has been inoculated with the test organism. (2) Counting techniques from which a death rate or time-survivor regression can be calculated. (3) Methods involving measurement of the respiration rate or oxygen uptake. (4) Turbidimetric methods which can be divided into two sub-groups: (a) Those measuring the extent of the inhibition of growth of the organisms in presence of the bactericide. (b) Those used for counting the survivors and from which death rate or time-survivor regressions can be calculated. (5) *In vivo* tests.

In all *in vitro* methods the following factors must be taken into consideration. (a) The concentration of the bactericide; (b) The temperature of the reaction; (c) The time of exposure of the bacteria to the bactericide; (d) The medium in which the reaction takes place; (e) The test organism used, and even the strain and its history prior to use; (f) The concentration of organisms used as an inoculum. Variations in one or more of these factors will affect the result, and in any accurate and dependable measurement all but one of these factors must be kept constant.

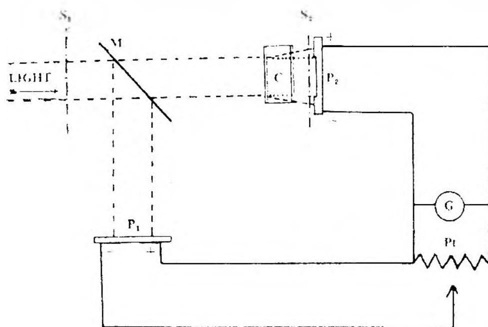
In this work the action of phenol on *Bacterium coli* was studied. The time of exposure of the bacteria to the bactericide was the variable, and a nephelometer was used to count the number of survivors, with the object of calculating a time-survivor regression and examining the reproducibility of such regressions.

EXPERIMENTAL

Nephelometer. The nephelometer used was the compensated photometric type described by Needham[1]. It consisted essentially of a collimated beam of light which was divided by interposing a sheet of optically flat, plain glass at 45°C. in this beam. The portion of the beam reflected from the glass fell on the compensating photoelectric cell (P_1); the other portion of the beam passed through a flow-through type of glass cell having two optically flat sides 3 cm. apart and a drain vent at the base to enable the bacterial suspension to be drawn off; the light

from this cell then fell on the second photocell (P_2). From the centre of the sensitive surface of this second photocell P_2 a disc, slightly larger in diameter than the light beam, had been removed, thus only light scattered by the suspension activated this second photocell. The output from this second photocell was connected across a reflecting galvanometer, whilst that of the first photocell was connected across a potentiometer, graduated 0 – 100, and thence with reversed polarity to the galvanometer.

Figure 1 is a diagrammatic sketch of the nephelometer and circuit.



$P_1 : P_2$	—Photocells.	$S_1 : S_2$	—Shutters.
G	—Galvanometer.	C	—Cell for suspension.
Pt	—Potentiometer.	M	—Sheet of plain glass.

FIG. 1.—Diagrammatic sketch of nephelometer

The apparatus was also fitted with two shutters S_1 in the main beam and S_2 in the beam incident on the second photocell.

Reference to the circuit diagram shows that the compensating photocell P_1 acts as a reference standard, and before any measurements could be taken this reference had to be set up at an arbitrary value, which, once chosen, had to be maintained throughout the whole work. To do this, the procedure at the beginning of any measurement was as follows:—(a) With both shutters closed the instrument was switched on and allowed 2 minutes for the light circuit to reach equilibrium. (b) A 66 per cent. transmission filter was placed in front of the photocell P_1 and the potentiometer dial was set at 75. (c) The main beam shutter was opened and the intensity of the light adjusted, by means of a variable resistance in the light circuit, until the galvanometer showed a deflection corresponding to 10 μamps. (d) The main shutter was closed and the filter removed.

During the course of an experiment checks were periodically made by isolating the photocell P_2, reinserting the 66 per cent. filter, setting the potentiometer at 75 and noting if the galvanometer reading still corresponded to 10 μamps.

The method of using the nephelometer once it was set up as described was:—(a) The shutter in front of photocell P_2 was opened. (b) The suspension whose turbidity was to be measured was poured into the cell. (c) The main beam shutter was opened—this caused the galvanometer

to swing according to the relative response of the two photosensitive surfaces. (d) The potentiometer was adjusted until the galvanometer showed no deflection, and this reading of the potentiometer was recorded. (e) The main beam shutter was closed and the suspension discarded. (f) The next suspension was run into the cell or, if no more readings were to be taken, the cell was sterilised by a 2 per cent. solution of phenol in alcohol and the instrument switched off.

Standard Capillary Dropping Pipettes. The pipettes were made and cleaned according to the method described by Withell[2] and Berry and Michaels[3], with the exception that an 0·5 per cent. Teepol solution was used in place of the 5 per cent. solution used by Berry and Michaels.

Throughout the experiments the use of tap water for washing glassware was avoided where possible, and, where any glass apparatus had been washed in tap water, at least three separate and distinct rinsings with distilled water always followed. In this way, it was found that the formation of the white scale, often noted on glassware after repeated use, washing and dry heat sterilisation, was considerably reduced. The pipettes, after cleansing in Teepol solution, were rinsed in hot distilled water, and dried in a warm oven. The tip of each pipette was examined. initially and after each experiment, under a X,2. magnifying lens and those with uneven or rough edges were rejected. The wide ends of the satisfactory pipettes were plugged with non-absorbent cotton-wool, packed in glass tubes, and sterilised by dry heat at 150°C. for 1 hour. After use, when the pipettes were contaminated with bacteria, they were placed in a flat enamelled or pyrex dish and autoclaved at 10 lbs. for 1 hour and then the cleansing cycle repeated.

Other Pipettes. The 25 ml., 20 ml., 10 ml., 5 ml., and 2 ml. pipettes used in the experiments were all normal laboratory grade B type; grade A were not used since repeated dry heat sterilisation would reduce the accuracy of such pipettes. When possible, pipettes with reasonably wide bore tips were chosen in order to keep the times for filling and emptying to a minimum. To facilitate the choice of the correct pipette, the wide bore ends of the pipettes were plugged with cotton-wool, a different coloured wool being used for each size of pipette. Sufficient pipettes for one experiment were packed in a copper tube 2 ft. × 3 in. diam. and sterilised by dry heat.

Medication Tubes. These were standard 50 ml. Pyrex glass boiling tubes with standard (B19 or B24) ground-glass stoppers.

Culture Bottles. 4-oz. flat-sided glass bottles closed by aluminium screw caps and fitted with rubber washers were found to be the most convenient for culture bottles.

An experiment was devised to compare the suitability of the glass-stoppered boiling tube (used as medicating-tubes) with that of 4-oz. screw-capped bottles as culture containers.

The original intention was to use 50 ml. of peptone broth for culture purposes, but as 50 ml. completely filled most of the glass-stoppered tubes, for the purpose of this experiment only 45 ml. of broth was used in each case.

(a) Experiment:—(i) 12 bottles and 12 tubes, each containing 45 ml. of sterile peptone broth, were placed in an incubator at 37°C. overnight. They were then divided into three groups, each group containing 4 bottles and 4 tubes.

(ii) Into the bottles and tubes of the first group were placed 5 drops of a 24-hour culture of *Bact. coli*, into those of the second group 10 drops of the same culture and into that of the third group 15 drops.

(iii) One tube and one bottle from each group were, respectively, incubated for 2, 3, 4 and 5 hours, and the turbidities then measured on the nephelometer.

(b) Results:—The results, shown graphically in Figure 2, illustrate that a difference does exist between bottles and tubes.

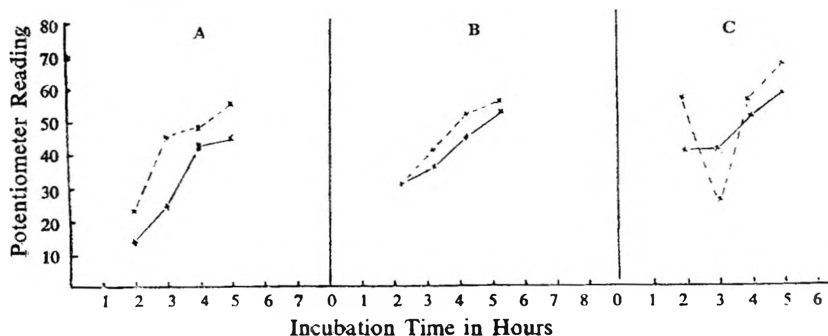


Fig. 2.—Showing comparison of turbidity produced when 5 drops (A), 10 drops (B) and 15 drops (C) of a 24-hr. culture of *Bact. coli* were incubated in bottles (broken lines) and tubes (continuous lines)

(c) Discussion:—The difference between bottles and tubes may be attributed to the greater volume of air above the culture medium in the bottles when compared with tubes, which permits of more rapid aerobic growth. The results clearly indicated the necessity for a standardised culture bottle. A sufficient quantity of 4-oz. bottles for the complete work was obtained. *p*H tests on distilled water autoclaved in these bottles indicated that considerable amounts of alkali were leached out of the glass and so the whole consignment of bottles was autoclaved with acid, followed by washing and double autoclaving with distilled water until no measurable amount of leaching was observed.

Withell[2] pointed out that there was reason to believe that freshly vulcanised rubber liberated substances which are bactericidal to some organisms and, to counteract this, all new rubber discs used in the culture bottles were autoclaved before use. The absorption by rubber caps, of phenol-like substances, which occurs on autoclaving was not a factor in the present series of experiments as the culture bottles were never autoclaved whilst containing any phenolic substances.

Culture Tubes. The test tubes employed for agar slopes were of pyrex glass 6 in. by $\frac{5}{8}$ in.

Materials. Liquid Medium. The importance of a uniform culture medium in bactericidal evaluation was stressed by Wright[4], who found

that different samples of peptone gave different phenol coefficients; this variation in peptones was also noted by Philbrick[5], and Brewer[6] suggested that the variation may be due to the effect of added substances on the phospholipide content of the peptone.

Needham[7] in his original paper on the nephelometer suggested the simple formula:—

"Oxoid" peptone	10 g.
Sodium Chloride (A.R.)	5 g.
Distilled water	1000 ml.

In his experiments with peptones, "Oxoid" peptone not only proved superior to five other varieties of peptone, but he also showed that, with the use of "Oxoid" peptone, very little advantage was derived from the addition of the customary meat extract. Experiments also showed that only slightly heavier growth resulted from the use of 2 per cent. peptone. The substitution of the mixture of salts employed in Ringer's solution for the sodium chloride in the suggested formula resulted in a precipitate on heating and necessitated a further filtration, and so was not substituted in the formula. The simplicity and ease of preparation of such a culture medium commended it, and it was used throughout these experiments. To eliminate any inter-batch variation in the peptone, as noted by Brewer, a large consignment of one batch of "Oxoid" peptone was kept exclusively for this work.

The method of preparation was to dissolve the solids in water (no heat is required if only 1 per cent. peptone is used); the *p*H was adjusted to 7·3 by the addition of N potassium hydroxide. A careful check was kept on the amount of potassium hydroxide solution added, and on no occasion did the solution require N potassium hydroxide outside the limits of $4{\cdot}0 \pm 0{\cdot}5$ ml. of medium. The broth was filtered and distributed by an Ayling filter into 4-oz. culture bottles. The broth was then sterilised by autoclaving at 10 lbs. for 20 minutes. On occasions, for easier storage purposes 5 litres of double-strength broth were made and diluted with freshly boiled and cooled distilled water and re-filtered before distribution and autoclaving.

A routine check of *p*H after autoclaving was carried out on a sample bottle from each batch of medium made. A regular drop of 0·1 units of *p*H was observed in almost every case.

Solid Media. 2 per cent. of powdered agar was added to some of the peptone water prepared as above, and warmed until the agar dissolved. The molten material was clarified by passing through washed paper pulp and distributed into tubes which were plunged and sterilised by autoclaving for 30 minutes at 10 lbs. Before the molten material had set, the tubes were removed from the autoclave and laid in a sloping position until the medium solidified. The tubes were stored in a dust-proof tin at room temperature.

Test Organism. 24 hour cultures of *Bact. coli* (Lister Institute No. 5933) were freeze-dried and the material distributed into a number of sterile tubes and sealed. These cultures were stored at room temperature.

At the beginning of each month a tube of freeze-dried material was opened and the contents suspended in a little sterile distilled water. Four agar slopes were inoculated with this suspension and incubated at 37°C. for 24 hours. These cultures were classed as master slopes, sealed with paraffin wax and stored at room temperature; no master slope was used if it had been stored for longer than 2 months.

A portion of the growth of one of the master slopes was removed with a sterile platinum loop and used to inoculate four fresh slopes; after 24-hours incubation at 37°C. these slopes were taken from the incubator and a small portion of the growth of each slope was used to sub-culture on 4 fresh slopes. This 24-hour subculturing was performed daily, and only slopes from the 4th to 14th subculture were used in the experiments.

Distilled Water. The water used throughout this work was distilled from a " Baracop " still, which, fitted with an efficient baffle to prevent entrainment, and a glass condenser, supplied a distilled water free from the metallic impurities noted by Davis[8] and Wilson[9,10].

Phenol. Standard 5 per cent. solutions in freshly boiled and cooled distilled water were made up each week from analytical grade phenol. Solutions of greater dilution were made up from these standard solutions just prior to each experiment.

Preliminary Investigations

Turbidity Response Curve. In his review of photoelectric instruments, Baier[11] points out that although nephelometers are sensitive to small amounts of turbidity the linearity between the intensity of light measured and the amount of suspended matter holds moderately well within narrow limits when the sample is faintly turbid, but with increasing turbidity the effect of secondary absorption may become so great as to give a lowering of the intensity of the scattered light. The following experiment was performed to examine the linearity of the dose response curve of the nephelometer.

Fourteen bottles, each containing 50 ml. of sterile peptone broth were taken; into these bottles, from the same sterile standard dropping pipette, were dropped 1, 2, 5, 10, 15, 20, 25, 30, 40, 50, 75, 90, and 110 drops respectively of a 24-hour culture of *Bact. coli.* Immediately the bottles were shaken and the turbidity measured on the nephelometer. The results were plotted on Figure 3.

These results showed that, assuming each drop of the 24-hour culture contained approximately the same number of bacteria, a linear relationship exists between nephelometer reading and number of bacteria for readings up to 80. The values of the nephelometer reading above 80 were below those anticipated by the straight line, dotted in Figure 3, and indicated that a little secondary absorption might be taking place.

Effects of Size of Sample and Period of Incubation. To measure these effects 35 culture bottles, each containing 50 ml. of sterile peptone broth were stored in an incubator until a constant temperature of 37°C. had been attained. They were then divided into 5 groups with 7 bottles in

each group; each group subsequently received the same treatment. Nil, 1 drop, 2, 4, 8, 16, and 32 drops, respectively, of a 24-hour culture of *Bact. coli* were dropped, from a standard dropping pipette, into the 7 bottles of each group. The groups were then incubated for 2, 3, 4, 5 and

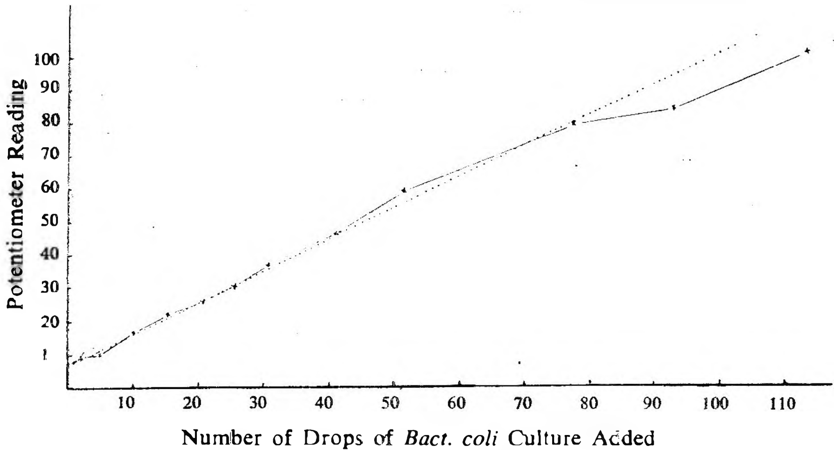


FIG. 3.—Turbidity Response Curve. Showing the turbidity produced when drops of a 24-hr. culture of *Bact. coli* were added to 50 ml. of peptone broth.

6 hours respectively at 37°C. At the end of the incubation period the turbidities of the suspensions were read on the nephelometer. Table 1 shows the results obtained.

It was anticipated that a time survivor curve for something less than 20 per cent. of survivors would be plotted, and the results of Table 1 would be for 100 per cent. of survivors.

TABLE I

TURBIDITIES (QUOTED AS POTENTIOMETER READINGS) PRODUCED WHEN VARIOUS NUMBERS OF DROPS OF A 24-HOUR CULTURE OF *Bact. coli* WERE INCUBATED, IN 50 ML. OF BROTH, FOR VARYING PERIODS

No. of Drops	Turbidities corresponding to incubation periods				
	2 hr.	3 hr.	4 hr.	5 hr.	6 hr.
0	5	6	8	7	6
1	19	18	58	87	98
2	9	31	78	95	97
4	13	51	87	89	B.S.
8	23	71	93	98	B.S.
16	46	85	95	B.S.	B.S.
32	71	94	97	B.S.	B.S.

B.S.—Beyond the scale of the instrument.

Wilson[9] established the fact, that, in using the standard dropping pipette for bacterial suspension measurement it was inadvisable to use less than 4 drops. Also, Alper and Sterne[12] found that the optical behaviour of the individual cells of a growing culture did not approach that of the parent culture until the culture was 6 to 7 hours old, and Huntingdon and Winslow[13] showed that the cell volume of individual cells in such a

culture of *Bact. coli* was the same as that of the parent culture after $5\frac{1}{2}$ hours' growth.

Thus the minimal time for incubation would appear to be 5 to 7 hours. Considering initial samples of 20, 10 or 5 drops then 20 per cent. survivors from these samples would correspond to 4, 2, or 1 drops respectively in Table 1. From a consideration of Table 1 for a 5-hour incubation period, a maximum survivor level of 5 per cent. could be measured for a 20-drop sample and 20 per cent. survivors for a 5-drop sample. For incubation periods of more than 5 hours only much smaller percentages of survivors would be readable.

Use of a Suspension of Bacteria. The variations in the opacity of a 24-hour culture of *Bact. coli* was unknown, and the opacity was too great for direct measurement on the nephelometer. A sample of such a culture could have been diluted and then measured on the nephelometer, and hence would have acted as a measure of the opacity of the original culture. This sampling would be necessary because the chances of contamination during a nephelometer reading were high and hence any sample used in the nephelometer must be discarded after measurement. With such a method of standardisation any 24-hour culture which showed more than standard opacity would have to be diluted with fresh sterile peptone broth until its opacity equalled that of a standard; and, as no method was available for increasing the opacity of any culture, then a standard would have to be chosen such that its opacity would be below that of any 24-hour culture; and each 24-hour culture would have to be diluted, maybe with considerable amounts of sterile peptone broth. The introduction of fresh sterile medium would result in renewed growth of the culture at any time before and perhaps also during the disinfecting period.

The idea of using a standardised 24-hour culture was discarded in favour of a standard suspension in a vehicle which did not encourage growth subsequent to standardisation.

Bean and Berry[14] have shown that Wilson's modification of Ringer's solution, the suitability of which in the majority of cases was proved by Berry and Michaels[3], should not be used in presence of soap solutions. Since a test of solutions of chloroxylenol in potassium ricinoleate was envisaged, Ringer's solution could not be used for the suspension and so distilled water was used.

Preparation of Suspension. The organisms from 3 of the current agar slope subcultures were washed off into a culture bottle containing 50 ml. of sterile water and a few glass beads. This suspension was shaken for about 1 minute to break up any clumps of organisms. Half (25 ml.) of this suspension was withdrawn and transferred to an empty sterile culture bottle, diluted with 25 ml. of distilled water and used in the nephelometer. The nephelometer was set up with a 50 per cent. transmission filter in the main light beam. The freshly prepared and diluted suspension was poured into the optical cell and both photocells switched in. The potentiometer setting for a zero galvanometer reading was noted. By a system of trial and error sufficient distilled water was measured into the

optical cell until the galvanometer gave a zero reading with the potentiometer set at 50. The amount of distilled water necessary was recorded and the suspension then discarded. From the amount of distilled water added to half the original suspension it was possible to calculate the amount of sterile water to be added to the remaining 25 ml. of suspension such that the suspension formed, if diluted with an equal quantity of water, would give a reading of zero on the galvanometer, with the potentiometer set at 50 if placed in the nephelometer with a 50 per cent. transmission filter in the main beam. Checks with a Burroughs Wellcome Opacity Set and a plate count indicated that such a suspension contained approximately 175 million organisms per ml. of suspension.

Comparison of Suspension and 24-hour Culture. To compare such a suspension with a 24-hour culture the following experiment was performed: Six culture bottles each containing 50 ml. of sterile peptone broth at 37°C. were taken. 1, 2, 4, 8, 16 and 32 drops of a trial suspension, respectively were dropped from a standard dropping pipette into the bottles and they were then incubated for 3 hours and their turbidities measured.

The experiment was repeated using a 24-hour culture in place of the suspension. The results are tabulated in Table II.

These results show that a suspension of *Bact. coli* of the stated opacity approximates in activity a 24-hour culture of the organism.

Reproducibility of Suspension. Further 3-hour incubation tests were performed on suspensions made for other experiments to investigate the

TABLE II

SHOWING THE TURBIDITIES PRODUCED AFTER 3-HOURS INCUBATION OF VARIOUS NUMBERS OF DROPS OF A SUSPENSION, AND 24-HOUR CULTURE OF *BACT. COLI*.

	Turbidity (measured as Nephelometer Readings)					
No. of drops ...	1	2	4	8	16	32
Suspension	15	45	47	84	89	96
24-hour culture ...	18	31	51	71	85	94

TABLE III

SHOWING THE TURBIDITIES PRODUCED AFTER 3-HOURS INCUBATION OF VARIOUS NUMBERS OF DROPS OF SEVERAL SUSPENSIONS OF *BACT. COLI*.

	Turbidity (measured as Nephelometer Readings)					
No. of drops ...	0	1	2	5	10	20
Experiment No.						
34	6	22	37	66	81	98
35	3	19	47	72	91	B.S.
36	4	27	44	65	80	92
37	5	20	32	54	75	90
38	6	26	45	65	80	97
39	12	29	54	76	88	B.S.
40	6	30	63	78	92	97
41	4	20	40	71	84	97
42	4	32	58	75	91	97

reproducibility of the suspension. Table III shows the results of these tests.

These results, whilst showing a fair measure of agreement, were not analysed, since the temperature during the 3-hour incubation period was not constant.

Bactericidal Value of Phenol

Reaction Time. The time during which the bacteria are in actual contact with the bactericide is called, throughout this work " reaction time."

Preliminary Experiments. Preliminary experiments had shown that with 0·75 per cent. phenol, reaction times of 30 to 50 minutes would produce turbidities within the nephelometer range.

Technique. Concentrations of phenol of 0·72 per cent., 0·74 per cent., 0·76 per cent. and 0·78 per cent. were chosen as the strengths for use in these investigations.

(1) 50 bottles each containing 50 ml. of sterile peptone broth were stored in an incubator at 37°C. until just prior to the experiment, when they were transferred to a water-bath at 37°C.

(2) A standard suspension of *Bact. coli* was prepared and placed in a water-bath at 20°C.

(3) Solutions of phenol of 3/2 times the test strength were prepared from a standard 5 per cent. solution by dilution with freshly boiled and cooled distilled water. When prepared, these solutions were placed in the water-bath at 20°C.

(4) 4 Sterile glass-stoppered medication tubes were placed in the water-bath. A sufficient length of time was allowed for all temperatures to reach a constant level.

(5) 10 ml. of the first phenol dilution was transferred by means of a sterile pipette to one of the medication tubes and then the tube returned to the 20°C. water-bath.

(6) At a time zero, 5 ml. of the standard suspension was added to the dilute phenol in the medication tube which was shaken and returned to the water bath.

(7) At a time (zero + t $-$ $\frac{1}{2}$) the medication tube was taken from the water-bath, shaken, and a sterile dropping pipette filled with the phenol-suspension mixture and the medication tube returned to the water-bath.

(8) At a time (zero + t) 20 drops were dropped from the pipette into one of the culture bottles containing the 50 ml. of sterile peptone broth, which was then returned to the water-bath at 37°C. The addition of drops was started 10 seconds before (zero + t) minutes, and by adding one drop per second, the mean time for the addition of the 20 drops was (zero + t.).

(9) Further samples of 20 drops of the reaction mixture were transferred to culture bottles at zero + t + 1, + 2, + 3, ... + 10 minutes respectively.

Operations (2) and (5)-(9) were conducted under aseptic conditions.

(10) The operations (5)-(9) were repeated for the other dilutions of phenol.

(11) The turbidities of the suspensions were read on the nephelometer after exactly 5 hours' incubation.

Results. The results of two consecutive experiments are shown in Table IV.

TABLE IV

SHOWING THE TURBIDITIES PRODUCED AFTER 5 HOURS INCUBATION OF 20 DROPS OF REACTION MIXTURES CONTAINING 0.72 PER CENT., 0.74 PER CENT., 0.76 PER CENT., AND 0.78 PER CENT. PHENOL.

	Turbidity (measured in Nephelometer Readings)									
0·72 per cent. Phenol.										
Reaction Time, minutes ...	50	51	52	53	54	55	56	57	58	59
Expt. No. 38	84	92	89	90	87	78	63	61	66	65
Expt. No. 39	82	83	98	79	77	70	68	70	73	70
0·74 per cent. Phenol.										
Reaction Time, minutes ...	40	41	42	43	44	45	46	47	48	49
Expt. No. 38	75	77	60	59	62	45	35	27	23	29
Expt. No. 39	66	64	62	62	61	58	39	40	37	30
0·76 per cent. Phenol.										
Reaction Time, minutes ...	30	31	32	33	34	35	36	37	38	39
Expt. No. 38	70	64	56	60	57	49	33	29	30	26
Expt. No. 39	93	90	85	86	76	74	73	66	63	64
0·78 per cent. Phenol.										
Reaction Time, minutes ...	20	21	22	23	24	25	26	27	28	29
Expt. No. 38	81	73	65	69	64	65	46	45	40	41
Expt. No. 39	B.S.	B.S.	99	98	96	93	89	83	80	78

Extrapolating in the case of 0·78 per cent. the turbidities at 30 minutes would be 37 in Experiment No. 38 and 75 in Experiment 39.

Thus we have for 30 minutes.

0·78 per cent. phenol in Expt. 38:— 37
Average 66
0·78 per cent. phenol in Expt. 39:— 75
0·76 per cent. phenol in Expt. 38:— 70
Average 82
0·76 per cent. phenol in Expt. 39:— 93

Thus the difference due to change of phenol strength is 16 and the difference due to daily variation (value in Expt. 39 – corresponding value in Expt. 38) is 38 for 0·78 per cent. phenol and 23 for 0·76 per cent. phenol. In this instance, the daily variation was greater than that due to phenol strengths; if then the experiments are to be used for differentiating between the different strengths of bactericides, this daily variation would have to be reduced. To eliminate the effect of the daily variation, the results for 28 experiments were averaged; these results, plotted in

Figure 4, showed a definite difference between the various strengths of phenol used, but insufficient similarity between the graphs to warrant a detailed analysis.

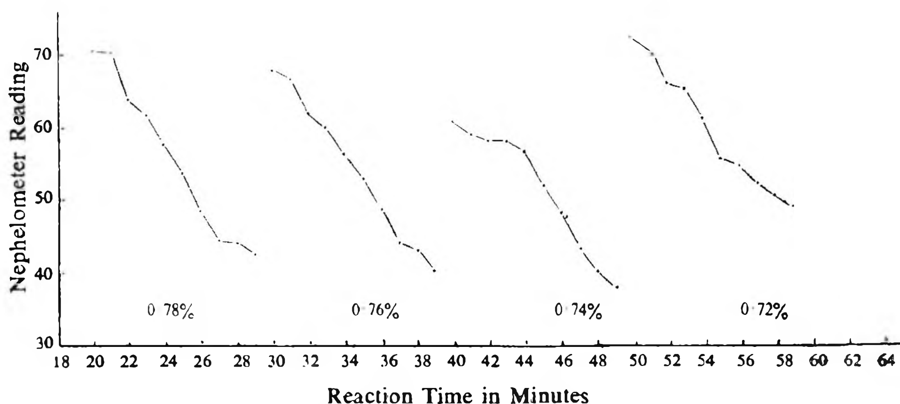


FIG. 4.—Showing the average turbidities produced using 4 different strengths of phenol for various reaction times.

DISCUSSION

Baier[7] has pointed out that with photoelectric nephelometers the linearity between the amount of suspended matter and nephelometer reading holds only for comparatively small amounts of suspended matter. The turbidity response curve plotted in Figure 3 shows that used under similar conditions linearity does hold for the nephelometer, although readings over 90 on the nephelometer should be treated with caution. Considering the findings of Alper and Sterne[8] and Huntingdon and Winslow[9] in connection with the changes in cell size and opacity of young cultures of *Bact. coli,* and having regard to the length of a normal working day and the result shown in Table I the optimal time in incubation appears to be 5 hours with the use of samples of a size less than 20 drops.

The reasons for use of a standard suspension of bacteria instead of a 24-hour culture have already been outlined.

Phenol was used as the bactericide in the preliminary tests because the day to day variations in phenol solutions would be very small and so permit of study of the variations due to experimental technique, and not in order that at a later date similar experiments could be performed with other bactericides and a form of " phenol coefficient " calculated.

The main conclusion to be drawn from the results of the experiments on the bactericidal value of phenol was that a large day to day variation was present. The results of the averages for 28 experiments showed that the method could be applied if some reduction of the daily variation could be accomplished. Table III shows that a small variation in the turbidities produced from the standard suspension does exist but this is not large enough to account for the variation in the final results. The

conclusion could then be drawn that the high daily variation was due, not to the variation in preparing the standard but to the treatment of the standard after preparation. This treatment could be divided into three parts: (i) reaction stage, (ii) incubation stage, (iii) measurement. Stage (i) was similar to the reaction stage in other methods of bactericide evaluation and it seemed doubtful if any further elaboration of this stage would reduce the variation. Thus further work on this method was concentrated on stages (ii) and (iii).

SUMMARY

1. Samples were taken, at predetermined time intervals, from a reaction mixture of phenol and *Bact. coli*, and inoculated into sterile peptone broth. The turbidity of these samples after incubation was measured with a nephelometer in an effort to plot a time-survivor curve.

2. The turbidity-response curve of the nephelometer was found to be a straight line. A 5 to 20 drop sample and a 5 hour incubation period proved to be the most advantageous.

3. The daily variation in the results showed that better control of incubation and turbidity measurement was needed before reproducible results could be anticipated.

REFERENCES

1. Needham, *Nature*, 1946, **157**, 374.
2. Withell, *Quart. J. Pharm. Pharmacol.*, 1938, **11**, 736.
3. Berry and Michaels, *Quart. J. Pharm. Pharmacol.*, 1947, **20**, 331.
4. Wright, *J. Bact.*, 1917, **2**, 315.
5. Philbrick, *Soap*, 1937, **13**, (2), 103.
6. Brewer, *Soap*, 1943, **19**, (4), 101.
7. Needham, *J. Hyg., Camb.*, 1947, **45**, 1.
8. Davis, *Quart. J. Pharm. Pharmacol.*, 1940, **8**, 14, 32.
9. Wilson, *J. Bact.*, 1922, **7**, 405.
10. Wilson, *Spec. Rep. Ser. Med. Res. Coun., London*, 1935, No. 206, 38.
11. Baier, *Physiol. Zool.*, 1947, **20**, 172.
12. Alper and Sterne, *J. Hyg., Camb.*, 1938, **33**, 497.
13. Huntingdon and Winslow, *J. Bact.*, 1937, **33**, 123.
14. Berry and Bean, 1949, *private communication*.

A NOTE ON THE OXIDATION OF PAPAVERINE BY SELENIUM DIOXIDE

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During the course of recent work, a supply of papaveraldine was required for the purposes of synthesis. The classical preparation of this compound is due to Goldschmiedt[1], who oxidised papaverine by potassium permanganate. Only a 53 per cent. yield of papaveraldine was claimed for this method, and it was therefore decided to investigate other methods of oxidation, selenium dioxide being finally chosen as the oxidising agent.

The most recent review of selenium dioxide oxidation[2] quotes only one example of the oxidation of papaverine by selenium dioxide, the method of Menon[3]. This author carried out the reaction in acetic acid solution, and gives no actual yield for the quantities used, although stating in the text that " The quantitative yield of papaveraldine and the absence of other degradation products is specially noteworthy." Postowsky and Lugowkin[4] carried out the oxidation of diphenylmethane to benzophenone in 87 per cent. yield by heating an intimate mixture of the hydrocarbon and selenium dioxide to 200° to 210°C., in the absence of a solvent, whereas Fisher[5], who used alcohol as a solvent, claimed only 47 per cent. yield of benzophenone. It seemed probable that the oxidation of papaverine by a modification of the method of Postowsky and Lugowkin would give satisfactory results, and this was therefore carried out.

Experimental

An intimate mixture of papaverine (15 g.) and selenium dioxide (6 g.) contained in a 250 ml. beaker was placed in an oil bath (oil temperature 180° to 190°C.) and thoroughly stirred. The mixture began to darken, soon fused, and a brisk reaction commenced, with the rapid evolution of steam. Steady stirring was required to prevent frothing. The chocolate-brown mixture then resolidified. It was heated for a total time of 30 minutes and allowed to cool; the brittle porous mass was powdered, and extracted with benzene (100 ml.) in a Soxhlet apparatus. When extraction was complete, the extract was cooled, filtered, washed with benzene (2 × 10 ml.) and dried, giving 14·99 g. of almost pure papaveraldine, m.pt. 204° to 206°C. The filtrate and washings, on evaporation, gave a reddish brown residue, m.pt. 192° to 202°C. (0·88 g.).

The main bulk was ground up with hydrochloric acid (30 ml. of concentrated acid in 100 ml. of water), the product made up to approximately 1 l. with water, heated to dissolve all the hydrochloride, filtered hot, and concentrated to small bulk under reduced pressure. On cooling, papaveraldine hydrochloride separated as fine yellow needles, m.pt.

200°C. (as recorded[3]). The crude material of m.pt. 192° to 202°C. also yielded a small quantity of papaveraldine hydrochloride. This was added to the main bulk, the whole dissolved in hot water, made alkaline with ammonia, allowed to cool, and the resulting papaveraldine filtered off, washed thoroughly with water, and dried. The product weighed 14·30 g. (91·6 per cent.) and melted at 210°C. (recorded 210°C.[1], 209° to 211°C.[3]).

SUMMARY

Papaveraldine has been prepared in 91·6 per cent. yield by the oxidation of papaverine with selenium dioxide.

I wish to thank the Directors of Messrs. Allen and Hanburys, Ltd., for permission to publish this paper, and Mr. C. J. Eastland for his co-operation.

REFERENCES

1. Goldschmiedt, *Mh. Chem.*, 1885, **6**, 956; 1886. **7**, 486.
2. Rabjohn, *Organic Reactions*, John Wiley and Sons, New York.
3. Menon, *Proc. Indian Acad. Sci.*, 1944, **19A**, 21 (*Chem. Abst.* **39**, 390 (1945)).
4. Postowsky and Lugowkin, *Ber. dtsch. chem. Ges.*, 1935, **68**, 855.
5. Fisher, *J. Amer. chem. Soc.*, 1934. **56**, 2056.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Cinchona, Limit Test for Quinine Content of. R. Moers. (*J. Pharm. Belg.*, 1949, **4**, 219.) The following colorimetric modification of the thalleoquinine test may be used to ensure that samples of cinchona contain the required minimum of 1·0 per cent. of quinine. The powdered bark is extracted by the method of the Belgian Pharmacopœia, and the solution is made up to 50 ml. and filtered. Portions of 1, 0·5, 0·25 and 0·125 ml. of this solution are made up to 5 ml. and treated with 0·4, 0·3, 0·2 and 0·1 ml. respectively of one-fifth saturated bromine water. After shaking for 10 seconds, 10 drops of solution of ammonia are added, and, after a further shaking for 15 seconds, the mixtures are diluted to 10 ml. with methyl alcohol. With 1 per cent. of quinine in the original bark, the smallest quantity gives no visible reaction; the thalleoquinine colour is first visible in the tube containing 0·25 ml. of the original liquid. The colours may also be determined photometrically, using a 440 filter. G. M.

Quinine and Quinidine, Differential Colour Reaction of. L. David. (*Pharm. Acta Helvet*, 1949, **24**, 427.) Since quinine and quinidine are stereo-isomers, they give in general the same reactions. They may be distinguished by means of the following reactions. 0·01 g. of the alkaloidal salt is mixed thoroughly, in a small basin, with 0·25 ml. of bromine water, and the mixture is immediately transferred to a test tube, and washed in with 1 ml. of water; 1 ml. of chloroform is added, and the mixture is allowed to stand for 3 minutes, shaking occasionally; the mixture is then treated with 1 drop of 10 per cent. potassium ferrocyanide solution, shaken, treated with 3 ml. of 5 N sodium hydroxide, and shaken again; the colour of the separated chloroform is observed by transmitted light, with quinine the liquid is colourless; with quinidine bright red. The two alkaloids may also be distinguished by a solubility reaction. 0·01 g. of the salt and 2 ml. of water are brought to the boil and treated with 1 drop of ferrocyanide solution; with quinine the solution is a first clear, then becomes milky, and a deposit of minute spherical particles forms on the walls of the tube, with quinidine a precipitate of yellowish needle crystals appears; as the deposit from quinine is amorphous, the polarisation microscope may be used to distinguish these two deposits. G. M.

ANALYTICAL

Eserine Solutions, Determination of Decomposition Products in. H. Hellberg. (*Svensk Farm. Tidskr.*, 1949, **53**, 638.) The deterioration of eserine solutions is initiated by decomposition of the urethane chain, leading to the production of methylamine, carbon dioxide and eserinol. The latter is oxidised to rubreserine and other products. The decomposition may be followed either by colorimetric determination of the rubreserine, or from the methylamine. From a solution of eserine, which has undergone some decomposition in presence of air, the unchanged eserine, together with some methylamine, may be extracted by means of ether, since the eserinol will have been oxidised to rubreserine, which is not extracted. Three methods

are proposed for the examination of such solutions. 1. A solution, containing 20 to 100 mg. of eserine salicylate in 10 ml., is made alkaline with sodium carbonate and extracted immediately with pure ether, using in all about 100 ml. of ether. The ethereal solution is dried with sodium sulphate, and evaporated to dryness, then evaporated down with more ether. The residue is dissolved in 0·01 N sulphuric acid, and the alkaloid is determined by back titration with borax, using methyl red as indicator. 2. The solution, containing 0·5 to 5 mg. of eserine, is extracted as before, the residue is dissolved in a few ml. of water and 3 drops of M sulphuric acid. After hydrolysis of the eserine, the methylamine is separated by distillation and determined colorimetrically with ninhydrin. 3. The solution, containing 0·5 to 5 mg. of eserine, is extracted as before. To the filtered ether solution 0·01 N sulphuric acid is added, and the solution is then evaporated on a water-bath. The eserine is then determined photometrically as rubreserine by the method previously described by the author (*Svensk Farm. Tidskr.*, 1947, **51,** 560). G. M.

Local Anæsthetics, Colorimetric Determination of. K. Steiger and F. Hippenmeyer. (*Pharm. Acta Helvet,* 1949, **24,** 443.) The method, based on the formation of reineckates, may be used for procaine, pantocaine and nupercaine, but not for panthesine. Adrenaline does not interfere. The reagent is prepared by shaking 1 g. of ammonium reineckate with 50 ml. of water for 10 minutes, and filtering. The determination is carried out as follows. The solution, containing 2 to 20 mg. of the local anæsthetic, is diluted to 8 ml., and treated with 1 ml. of 20 per cent. sulphuric acid and 4 ml. of reagent. After shaking vigorously, the mixture is allowed to stand for 1 hour, and then filtered through a sintered glass filter, the residue being washed with 5 ml. of water. After sucking dry on the pump, the precipitate is dissolved in acetone to 10 ml. and the colour is determined The absorption maximum is at 530 mμ, and a filter S 53 may be used.

G. M.

Morphine, Determination of, in Poppy Capsules. M. Mascré and C. Genot-Boulanger. (*Ann. pharm. franc.*, 1949, **7,** 493.) The following method is suitable for an accurate determination. Moisten 100 to 200 g. in coarse powder with 10 per cent. sodium carbonate solution or with methyl alcohol. Exhaust by percolation with methyl alcohol for 5 or 6 hours in a Soxhlet apparatus, concentrate to about 20 ml. and evaporate to a firm extract. The extract can be weighed and the proportion of morphine determined by the official method, but this involves measuring the moisture content of the lime extract and making corrections for it, which can be avoided as follows. Mix the extract with 1 g. of slaked lime and 20 to 25 g. of water, triturate carefully and transfer, with the aid of 3 quantities, each of 3 to 5 ml. of water, to a stoppered bottle. Allow to stand for 1 hour, shaking gently at frequent intervals. Filter with slight suction and, to the residue on the filter, add 2 to 3 ml. of lime water, and allow to stand for 10 to 15 minutes, stirring several times before filtering. Repeat the treatment of the residue on the filter twice and place the combined filtrates in a tared vessel. Take 25 g. of precipitate, titrate the morphine by the method of the French Codex and calculate the total quantity of morphine in the sample. Methyl alcohol is chosen as the menstruum to extract the capsules because it yields an extract which is compatible with milk of lime; chloroform, acetone, ethyl acetate and ethyl alcohol do not. For the purpose of selecting seed from plants of high morphine content, a quick, simple method giving an approximate assessment of morphine

content, and using a sample of as little as 5 g. is required. A suitable method is to remove the seeds, and extract 5 g. of powdered capsules by heating under reflux with methyl alcohol. An aliquot quantity of the extract, equivalent to 4 g. of capsules, is evaporated, and the morphine extracted with milk of lime or barium hydroxide solution. The solution is rendered acid by the addition of dilute acetic acid and treated with diluted Mayer's reagent to determine the quantity of reagent required to precipitate the alkaloid completely. The reagent is standardised in terms of morphine by repeating the experiment with graded quantities of morphine hydrochloride solution acidified with dilute acetic acid. The determination is only approximate, and is affected by changes in temperature. It is especially simple if it is only required to verify that the capsules contain at least, say, 0·5 per cent. of morphine. G. B.

Orange Oils from Palestine. H. T. Islip and F. Major. (*Bull. imp. Inst.*, 1948, **46**, 213.) Continuing an investigation of Palestinian sweet orange oils (*Bull. imp. Inst.*, 1947, **45**, 15) the authors report upon an examination of 9 further samples. Analytical figures for these are compared with those given by Nicholls, Parry and the British Pharmaceutical Codex. Only two of the samples conformed to the requirements of the B.P.C. in all respects. Regarding the aldehyde content for which there are no limits in the B.P.C., the figures obtained were in good agreement with those found previously at the Imperial Institute for Palestinian oils but none reached the minimum of the limits of Parry or Nicholls. The method employed for this determination was that of the Essential Oils Sub-Committee of the Society of Public Analysts, while Nicholls used a more drastic method using N hydroxylamine hydrochloride at a temperature of 60°C. The authors are of the opinion that higher results would be expected under these more drastic conditions, but it is open to question whether they are more accurate.

	$d^{15.5}_{15.5}$	$\alpha^{20°}_{D}$	$n^{20°}_{D}$	Aldehydes as decylic per cent.	Non-volatile matter per cent.
Sample 1	0·8500	+97·77°	1·4739	1·04	2·94
2	0·8536	+94·47°	1·4750	1·37	6·68
3	0·8514	+94·76°	1·4749	1·34	4·78
4	0·8502	+97·84°	1·4741	1·04	2·87
5	0·8504	+97·10°	1·4740	1·13	4·18
6	0·8510	+97·37°	1·4741	1·24	4·21
7	0·8506	+97·32°	1·4742	1·26	4·20
8	0·8542	+95·55°	1·4749	1·34	6·47
9	0·8522	+96·35°	1·4741	1·29	5·53
Palestinian Oil (Nicholls) ...	0·850–0·852	+97° to +99°	1·473–1·4745	1·5–1·9	3·2–3·8
Sweet Orange Oil (Parry) ...	0·848–0·854	+94° to +99°	1·4725–1·4750	1·5–3·0	1·5–4·0
Sweet Orange Oil (B.P.C.)	0·848–0·852	+95° to +99°	1·472–1·474	—	2–4

G. R. A. S.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

***Adenium Hongkel*, new Digitalis Heteroside from.** M. Frèrejacque and V. Hasenfratz. (*C. R. Acad. Sci., Paris*, 1949, **229**, 848.) A number of species of *Adenium* are used by the natives of Africa for arrow poisons. From the wood there was obtained a new heteroside, Hongkeline, which gave the reactions of digitalis glucosides. The formula is $C_{30}H_{46}O_8$ and it has m.pt. 130° to 135°C., $[\alpha]^{D}_{20°}$ $-10{\cdot}3°$ (in methyl alcohol). It contains a methoxyl group and has a lactone function. By acetylation there was obtained a diacetyl derivative, m.pt. 207°C. Hydrolysis gave a reducing sugar and a genin which could not be crystallised. G. M.

Phenylosazones, Identification of, by Chromatography. P. F. Jørgensen. (*Dansk Tidsskr. Farm.*, 1950, **24,** 1.) Although the phenylosazones of hexoses, pentoses, methylpentoses and tetroses with unbranched carbon chains cannot be completely separated by means of a single chromatographic column, this is possible by using a series of columns. The adsorbents used consist of calcium carbonate of definite particle size, and talc, the solvents being either chloroform containing a small proportion of alcohol (94 per cent.) or a mixture of equal volumes of acetone and chloroform. The first separation from chloroform-alcohol (3 per cent.) on calcium carbonate (8μ) gives a series of distinct bands, comprising, from the top downwards: sorbose; glucose and galactose; altrose: xylose, arabinose and rhamnose; fucose; and erythrulose. The first band is reworked from chloroform-alcohol (5 per cent.) on calcium carbonate (8μ), and gives two bands, the first of sorbose and glucose, the second of galactose. Sorbose and glucose are reworked from acetone-chloroform on talc, and give an upper band of sorbose and a lower one of glucose. The arabinose-rhamnose mixture from the first column is treated, from chloroform-alcohol (2·5 per cent.), on a column of calcium carbonate (3·5μ), and gives an upper band of arabinose and a lower one of rhamnose. The method may be applied to glycosides as follows: the compound is dissolved in alcohol (60 per cent.) and refluxed for 5 hours under reflux with dilute sulphuric acid. The alcohol is removed by evaporation, and, after cooling, the mixture is neutralised with barium hydroxide (methyl red). After filtration and decolorisation with charcoal, equal part of phenylhydrazine hydrochloride and sodium acetate are added. The mixture is heated for 15 minutes on the water-bath, filtered and cooled. The separated phenylosazone is dried and subjected to chromatographic analysis. The method can be used as a micro method, in which case the phenylosazone must be extracted from the reaction mixture with chloroform. An example of this is the application to digitoxose, the phenylosazone of which appears between the bands of arabinose and of rhamnose and fucose.

G. M.

ORGANIC CHEMISTRY

C^{14} Uniformly Labelled Fructose, Preparation of, by means of Photosynthesis and Paper Chromatography. S. Udenfriend and M. Gibbs. (*Science*, 1949, **110,** 708.) Four trifoliate bean leaves were allowed to photosynthesise in the present of 2·8 mc. of $C^{14}O_2$, and extracted with 80 per cent. alcohol. Polar compounds were removed by ether extraction and by passing through ion exchange columns (Amberlite 100-H and Duolite A-4) and hydrolysis of the sucrose was accomplished by heating with sulphuric acid at 80°C. for 10 minutes. The acid was removed by ion-exchange columns, and most of the glucose was removed by crystallisation on the addition of carrier glucose and alcohol. The fructose was separated from the remaining glucose by paper chromatography on 72 strips of filter paper (14 inches × 1½ inches). The chromatograms were developed with phenol and the bands were located by their radio-autographs. The fructose bands were cut out and extracted with 80 per cent. alcohol in a Soxhlet apparatus. The alcohol was removed by vacuum distillation, water being added from time to time, and traces of phenol were removed from the aqueous solution by ether extraction. After adding 30 mg. of carrier fructose, the solution was purified by passing through ion exchange columns and evaporated to a syrup at 35°C. *in vacuo*. Crystallisation was carried out in a centrifuge tube, 30 mg. of carrier fructose being added during the process. The purity of

the fructose was verified chromatographically, and microbiological degradation showed it to be uniformly labelled. The specific activity of the final product was 1·2 mc. per mg. of fructose. G. B.

TOXICOLOGY

Morphine, Diamorphine, Codeine and Barbiturates, Separation and Determination of Mixtures of. A. Stolman and C. P. Stewart. (*Analyst*, 1949, **74**, 543.) Separation by adsorption on a florisil column was investigated. All three alkaloids could be adsorbed under the conditions given but differential elution was difficult. Selective solvents were sought, and it was found that acetone would elute codeine and diamorphine but not morphine, whereas ethyl acetate would elute codeine but not morphine or diamorphine when these alkaloids were present alone. When the three alkaloids were present on the column together or in pairs however, these solvents gave no differential elution. Under the varying conditions tried it was not possible to effect complete separation of the alkaloids. Experiments with barbitone and phenobarbitone using florisil columns and acid or alkaline solutions similar to the model tissue extracts from which the three alkaloids had been adsorbed, showed that there was no adsorption of these barbiturates, thus making it possible to separate a mixture of these alkaloids from barbiturates. Removal of the barbiturates from the eluate of the florisil column was obtained on a column of activated cocoanut shell charcoal ground to 60 to 100 mesh particle size. Barbitone and phenobarbitone added to model solutions containing water, salt, alcohol, and trichloracetic acid were adsorbed over a wide range of *p*H (5·5 to 8·5), but ethyl acetate, in concentrations of only 6 ml. /100 ml. completely inhibited the adsorption. The adsorbed barbiturate was eluted by refluxing for 1 hour with ethyl acetate, and the eluate carefully evaporated to dryness. The crystalline residue was taken up in 10 ml. of water acidified with hydrochloric acid and the barbituric acid extracted from this by ether, which was then evaporated to dryness and the residue used for colorimetric determination by the cobaltous acetate-*iso*propylamine reaction of Levvy. Almost complete recoveries of barbiturates from model solutions could be obtained but urine determinations were not attempted owing to the pigment interference. It is considered that more detailed work is needed before the method can be accepted as of general value in toxicological analysis, although such further work may be undertaken with reasonable hope of success. R. E. S.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

***Actinomyces griseus*, a new Antibiotic from.** F. Grumbach, P Goret, E. Arquié, F. Boyer, C. Flachat and P. Villmin. (*C.R. Acad. Sci., Paris*, 1949, **229**, 787.) While acid extraction of the mycelium of *Actinomyces griseus* gives an antibiotic having the properties of streptomycin, alkaline extraction gives, in large yield, another substance which is very active towards Gram-positive organisms and streptomycin-resistant *Staphylococcus aureus*, but inactive towards *E. coli*, and only slightly active towards *Klebsiella pneumoniæ*. This substance is only found in certain strains of the organism, and is not present in any quantity in the

culture medium. It has no activity, *in vivo*, on mice infected experimentally with streptococcus or pneumococcus; and *in vitro* the inhibitory action is destroyed by the addition of serum to the culture medium. This antibiotic does not dialyse to any extent, will not pass through an L3 candle, and does not diffuse into gelose; thus it has a high molecular weight. G. M.

Aureomycin Hydrochloride, Adsorption on Aluminium Hydroxide Gel. F. E. Di Gangi and C. H. Rogers. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 646.) When aluminium hydroxide gel is added to a solution of aureomycin hydrochloride and allowed to settle, the supernatant liquid is colourless and the golden yellow colour is associated with the aluminium hydroxide gel. 5 ml. of aluminium hydroxide gel is capable of adsorbing almost all the aureomycin hydrochloride from 50 ml. of a 0·1 per cent. w/v solution. Experimentally, the solution before and after treatment with aluminium hydroxide gel, can be assayed bacteriologically, or colorimetrically using a Fisher electrophotometer with a filter having a band of approximately 425 mμ. Discrepancies between the two methods are due in part to deviation from the Lambert-Beer law in concentrations below 20 μg. per ml. 0·1 per cent. solutions of aureomycin hydrochloride are stable when stored for 8 weeks in a refrigerator, but weaker solutions lose some of their activity.

G. B.

BIOCHEMICAL ANALYSIS

Androsterone and Testosterone, Spectrophotometric Determination of. P. E. Hilmer and W. C. Hess. (*Anal. Chem.*, 1949, **21**, 822.) The method is based on the determination of the absorption spectra of the 2:4-dinitrophenylhydrazones of the hormones after treatment with 0·1N alcoholic potassium hydroxide. For the preparation of the hydrazones, 30 mg. was dissolved in 50 ml. of redistilled aldehyde-free ethyl alcohol and refluxed for 2 hours with 10 ml. of a saturated alcoholic solution of 2:4-dinitrophenylhydrazine, 1 ml. of concentrated hydrochloric acid was added and refluxing was continued for 2 minutes more. Distilled water was added until cloudy, the solution was cooled to room temperature, and was then placed in the icebox until precipitation was complete, the crude hydrazone being filtered off and washed with 95 per cent. ethyl alcohol and water. The hydrazone was dissolved in a minimum amount of benzene, adsorbed on a column (100 × 15 mm.) of alumina and treated with approximately 100 ml. of a 12 per cent. solution of chloroform in benzene, which resulted in the formation of two bands, the band of unreacted hydrazine being eluted by this solvent. The other band (hydrazone) was eluted with approximately 150 ml. of chloroform. Solutions of the hydrazones in chloroform, 2·5 ml. containing approximately 120 and 104 μg. of hydrazone or 74 and 64 μg. of hormone per ml. respectively, were treated with 5 ml. of 0·1N alcoholic potassium hydroxide, diluted to a volume of 50 ml. with alcohol (95 per cent.) and the ultra-violet absorption spectrum examined in a photoelectric spectrophotometer. The hydrazone of androsterone showed maximum absorption at 430 mμ.; the maximum for testosterone hydrazone was at 460 mμ. As an experiment in the separation of androgens from œstrogens a benzene solution containing *ca.* 20 μg. of each of the hydrazones of androsterone, testosterone, œstrone, and progesterone was adsorbed on a 100 mm. column of alumina, the column was washed with 10 ml. of benzene, 50 ml. of 1 per cent. acetone in light petroleum, and finally with chloroform until the washings were colourless. After evaporation of the solvent, the residue was dissolved in benzene, adsorbed on a 300-mm. column of florisil, washed with 5 ml. of benzene and then eluted with approximately

150 ml. of 20 per cent. solution of acetone in light petroleum. The androgens were eluted in the first portion of the acetone-petroleum washing, the solvent was evaporated, the residue was dissolved in 1·25 ml. of chloroform and made to 25 ml. with alcohol (95 per cent.). A 2 ml. aliquot portion of this solution was treated with 8 ml. of 0·1N alcoholic potassium hydroxide and the absorption taken at 430 and 460 mμ; standard solutions of androsterone and testosterone hydrazones were also treated with alcoholic alkali and the absorption taken at 430 and 460 mμ. The concentration of androsterone and testosterone in the "unknown" solution were then calculated from the formulæ of Knudson (*Ind. Engng. Chem. Anal. Ed.*, 1940, **12,** 715). Relatively good recovery of the hormones was obtained in five experiments by this method; the procedure was also used for preliminary studies on the determination of androgens in blood. R. E. S.

Aureomycin in Serum, Fluorimetric Determination of. J. C. Seed and C. E. Wilson. (*Science,* 1949, **110,** 707.) Silica gel, in No. 200 powder is washed with a current of distilled water to remove the very small particles, giving a suspension of particles of fairly uniform size which is used to prepare the adsorption columns. 1 ml. of the serum under test is passed through a column, followed by 1 ml. of isotonic saline solution to wash out the serum, and 1 ml. of alcohol to intensify the fluorescence. When the column is viewed in radiation from an argon-mercury lamp with a filter absorbing all wavelengths greater than 400 mμ, a band of yellow fluorescence due to the aureomycin is observed. The concentration of aureomycin in the serum may be assessed by comparing the width and intensity of the yellow fluorescent band with that in other columns, prepared in the same way, from serum to which measured quantities of aureomycin hydrochloride have been added. The presence of sulphonamides, penicillin, dicoumarol, salicylates, vitamins, streptomycin or chloramphenicol does not interfere with the determination. The fluorimetric method gives higher results than a bacteriological test, indicating that there is probably a substance present which is allied to, and associated with, aureomycin and which is fluorescent, but inactive bacteriologically. G. B.

Benzylpenicillin, Colorimetric Determination of. G. E. Boxer and P. M. Everett. (*Anal. Chem.*, 1949, **21,** 670.) A colorimetric method for the determination of benzylpenicillin in samples of any purity and in fermentation liquors is described and is based on the analytical separation of the active penicillin followed by the colorimetric determination of the phenylacetyl side chain of penicillin G. The substances used to stimulate the production of penicillin G in fermentation liquors are usually either basic or neutral phenylacetyl derivatives and extraction of the penicillins from the broth at low *p*H into an organic solvent and then re-extraction into a neutral aqueous phase, will effectively separate them from the precursor material. Two types of phenylacetyl derivatives (a) benzylpenicilloic acid and (b) various degradation products, are encountered in addition to benzylpenicillin itself. Penicillin G can be separated by chloroform extraction at *p*H 2 when the dicarboxylic acids (penicilloic acids) are not extracted. A blank obtained by conversion of the penicillin to penicilloic acid by treatment with either alkali or penicillinase gives a quantitative measure of any phenylacetyl-like substance other than benzylpenicillin extracted by chloroform at *p*H 2. In determinations of benzylpenicillin, the recrystallised sodium salt is extracted with chloroform and a glycine buffer at *p*H 2. The dried chloroform solution is evaporated and the residue nitrated with sulphuric acid containing 10 per

cent. of potassium nitrate (1 ml.) for 30 minutes on a steam bath. Following nitration and cooling, 2 ml. of water followed by 2 ml. of ammonia solution (sp.gr. 0·9) are added. The colour is developed by the addition of 2 ml. of a 15 per cent. aqueous solution of hydroxylamine and the optical density measured at 580 mμ in a suitable spectrophotometer using distilled water as a blank. A plot of the optical density against the amount of penicillin is linear. Fermentation broths should be diluted to contain 25 to 150 μg. of penicillin per ml. and extracted with amyl acetate and glycine buffer solution. The amyl acetate solution is extracted with ice-cold sodium phosphate solution (0·15 M) and the active solution is prepared by extracting the phosphate solution with hydrochloric acid (0·35 N) and ice-cold chloroform and treating it as above. The blank is prepared by heating the phosphate extract with (1·3 N) sodium hydroxide solution for 1 minute, adding hydrochloric acid (2 N) and extracting with chloroform. The recovery of penicillin added to unfermented broth was 95·5 ± 3·0 per cent. from fermented broth containing penicillin and at various stages of fermentation the analytical recovery of added penicillin over the amount already present was 97 ± 5 per cent. with largest deviation of -13 and +12 per cent. Total penicillins and penicillin G can be determined simultaneously. In the blank sample, penicillin is destroyed with penicillinase, hydroxylamine solution is added to both samples followed, after 3 minutes, by 1 ml. of a 20 per cent. solution of ferrous ammonium sulphate in (3·5 N) sulphuric acid and the developed colour read on a suitable spectrophotometer at 515 mμ with the blank as zero reading. Beer's law is obeyed over the entire range. The colour is essentially constant within the first 5 minutes and then decreases at the rate of 8 per cent. every 10 minutes. The hydroxamic acid formation is completed in 2 minutes and the compound is stable for as long as 2 hours. The recovery at 2 different levels of penicillin was 97±5 per cent. and the largest deviation −13 and +11 per cent. For intermediates in the purification, the accuracy was 99·4±4·4 per cent. with deviation of +6·3 and −8·4 per cent. R. E. S.

Citric Acid, Fluorimetric Determination of. E. Leininger and S. Katz. (*Anal. Chem.*, 1949, **21**, 810.) The method proposed is based on the fact that aconityl chloride is formed when anhydrous citric acid and anhydrous sodium carbonate are refluxed with thionyl chloride; after excess of thionyl chloride is volatilised, aconitamide is formed by exposure of the aconityl chloride to ammonia gas at room temperature followed by treatment of the aconitamide residue with 76 per cent. sulphuric acid at 165°C. to produce citraxinic acid. After neutralisation with ammonia an intense blue fluorescence is shown in ultra-violet light. A solution (1 ml. or less) of the sample containing 10 to 75 μg. of citric acid in a 25-ml. reaction flask is heated for 2 hours in a vacuum oven at 65° to 70°C. in order to obtain an anhydrous residue, and approximately 15 mg. of anhydrous sodium carbonate and 2 ml. of thionyl chloride are added to the sample. A combined reflux condenser and drying tube containing dehydrite is attached to the reaction flask which is heated in an oil bath at 95° to 100°C. After refluxing for 20 minutes, the reaction flask with the tube attached is removed from the oil bath and the excess of thionyl chloride is volatilised and evacuated through a three-way stopcock attached to the top of the condenser. The reaction flask is evacuated for 4 minutes after the residue appears dry and then air is allowed to flow into the flask by means of a capillary inlet through the three-way stopcock. The evacuation and introduction of dry air are repeated 3 times and the flask and condenser are introduced into an ammonia chamber. After removal of the condenser the residue in the

flask is exposed to ammonia for 10 minutes and then moistened with 2 ml. of 76 per cent. sulphuric acid. The flask is then heated for 6±0·5 minutes at 162° to 168°C. in an oil bath; after removal from the oil bath, the solution is diluted with 5 ml. of water and transferred quantitatively to a 100-ml. glass stoppered volumetric flask, the solution is made alkaline to litmus with dilute ammonium hydroxide (6N), made up to volume and the fluorescence intensity is measured in a fluorimeter at 24°±0·5°C. The effect of changes in various operating conditions at each step in the procedure is discussed and a detailed description of the apparatus is given. It is claimed that the method is applicable to the determination of 10 to 75 µg. of anhydrous citric acid; sulphate ions and hygroscopic substances interfered with the determination, although tartaric acid and malic acid up to 100 µg. did not. The application of the method to the determination of citric acid in citrus juices is described and the results are compared with those obtained by the A.O.A.C. method. R. E. S.

Panthenol, Microbiological Assay of. E. De Ritter and S. H. Rubin. (*Anal. Chem.*, 1949, **21**, 823.) The microbiological assay method for the determination of panthenol (αγ-dihydroxy-*N*-(3-hydroxypropyl)-ββ-dimethylbutyramide, the biologically active hydroxy analogue of pantothenic acid) due originally to Walter (*Jubilee Vol., Emil Barell* (Hoffman-La Roche, Inc., Basle) 1946, 98) has been modified to permit rapid assays of panthenol in the presence of pantoyl lactone as well as pantoic acid. The lactone is removed quantitatively from an aqueous test solution by continuous extraction for 1 to 2 hours with ethyl ether. The remaining aqueous solution is assayed for pantoic acid before and after alkaline hydrolysis the results indicating respectively, preformed and total pantoic acid; the difference represents pantoic acid formed by hydrolysis of panthenol. To correct for slight losses in the ether extraction a standard solution of panthenol is subjected to the same treatment. Pantothenic acid if present in relatively small amounts (up to 10 per cent. of the panthenol) will not interfere seriously with the panthenol assay since a partial correction for the pantothenate is provided by assay before hydrolysis. The assay medium of Sarett and Cheldelin (*J. biol. Chem.*, 1945, **159**, 311) using *Acetobacter suboxydans* was modified by the introduction, among other ingredients, of untreated liver concentrate, enzyme-digested casein, tween 80, and lactate. With the modified medium turbidimetric readings could be taken after 40 hours in stationary 50-ml. conical flasks or after 20 to 24 hours in colorimetric tubes if the latter were shaken continuously. Good agreement with bio-assays in rats was shown. R. E. S.

Vitamin B_1, Determination of. H. Utiger. (*Bull. Soc. Chim. biol.*, 1949, **31**, 238.) The determination of vitamin B_1 by its effect on the growth of the fungus *Phycomyces bl.* (by weighing the mycelium) does not give satisfactory results owing to interference by other compounds. The method proposed by the author is based on his observation that there is optimal accumulation of pyruvic acid at a concentration of vitamin of 0·04µg. per 20 ml., this being independent of time under the conditions given. Thus it is only necessary to determine the amount of an extract which gives the optimum production of pyruvic acid in order to determine the vitamin content. The culture solution consists of 20 ml. of a solution containing 3 per cent. of glucose, 0·1 per cent. of asparagin, 0·15 per cent. of potassium dihydrogen phosphate, and 0·05 per cent. of magnesium phosphate. The glucose solution is first treated with active carbon. The material to be examined is heated for 1 hour in an autoclave with 5N hydrochloric acid, the extract being then

filtered and adjusted to *p*H 6·5. Various amounts of the extract are added to 20 ml. of the nutrient solution, the mixture is sterilised, and finally inoculated with the spores of the fungus, and kept at 26° ± 1°C. for 96 hours. In order to determine the pyruvic acid the solution is filtered, and made up to 20 ml. A portion of this solution is made up to 2·0 ml. and treated with 1 ml. of a 0·1 per cent. solution of 2:4-dinitrophenylhydrazine in warm 2N hydrochloric acid. After standing for 10 minutes, the liquid is extracted with ethyl acetate 3 times, using only 1 ml. of the latter. The ethyl acetate is extracted with 3 quantities, each of 2 ml., of 10 per cent. sodium carbonate solution, the latter being washed with ethyl acetate. Five ml. of the aqueous solution is treated with 4 ml. of N sodium hydroxide and, after 10 min., the colour is determined photometrically using filter S50 and a 1 cm. cell. The quantity of pyruvic acid in µg. is then equal to Ek × 75·0. For the determination of the vitamin, a series of such tests are carried out with varying quantities of the extract under examination. At a certain concentration of extract, there is a maximum concentration of pyruvic acid, and this corresponds to 0·04 µg. of vitamin B per 20 ml. of nutritive solution. G. M.

PHARMACY

DISPENSING

Calcium Gluconate and Saccharate, Injection of. H. Siegrist. (*Pharm. Acta Helvet.*, 1949, **24**, 430.) Comparative trials of calcium lævulinate and calcium saccharate showed that the latter was the more effective means for increasing the solubility of calcium gluconate. Formulae are given for two solutions. Calcium gluconate injection: 80 ml. of freshly distilled water is boiled, and first 0·36 g. of calcium *d*-saccharate and then 9·5 g. of calcium gluconate is dissolved in it. After cooling, the solution is made up to 100 ml. with freshly boiled and cooled water, filtered, and filled into previously sterilised ampoules of hard glass. The ampoules are sterilised in the autoclave at 120°C. for 20 minutes. The sterilisation is repeated twice at 24-hour intervals. During the sterilisation the ampoules are laid on their sides. Strong injection of calcium gluconate: this is made similarly, and contains 0·72 g. of calcium *d*-saccharate and 19·0 g. of calcium gluconate in 100 ml. G. M.

Eserine Solutions, Stability of. H. Hellberg. (*Svensk farm. Tidskr.*, 1949, **53**, 658.) Although solutions of eserine readily assume a strong red colour, this does not necessarily indicate any considerable amount of decomposition. The first stage of the decomposition is a hydrolysis with formation of eserinol, which then oxidises to rubreserine. Thus even in the absence of air the solution decomposes, although it does not show any red colour. The addition of sulphite prevents the development of colour, but not the decomposition, since the sulphite merely converts the coloured products to substances which are colourless at an acid *p*H value. Solutions of eserine are fairly stable if the *p*H is not above 6; a solution in 2 per cent. boric acid may be kept for several weeks without any appreciable amount of decomposition other than a slight pink colour. The addition of sulphite, to prevent discolouration, is liable to lower the *p*H value, and is therefore undesirable for eye drops. Solutions of eserine must not be sterilised by heat, whatever the *p*H value. G. M.

Penicillin and Lanoline Ointments, Stability of. N. Å. Diding and E. Sandell. *Svensk farm. Tidskr.* 1949, **53,** 617.) The stability of penicillin ointment, prepared with anhydrous lanoline, depends on the state of oxidation of the latter. The formula used gave a product containing 1000 units of sodium penicillin per g., in a base of 9 parts of soft paraffin with 1 part of anhydrous lanoline. Eight samples of lanoline were used, these having peroxide values (ml. of 0·01 N sodium thioulphate per g.) ranging from 0·71 to 8·2. With 5 samples, where the peroxide value was not more than 1·24, the loss of penicillin activity was about 24 per cent. in 3½ months, the others (peroxide values 4 to 8) showed a loss averaging 58 per cent.

G. M.

Penicillin G, Pharmaceutical Applications of Aqueous Solutions of. G. Schuster, M. Dessus, J. Roux-Delimal and A. Morel. (*Ann. pharm. franc.*, 1949, **8,** 535.) The stability of ointments prepared by absorbing aqueous solutions of penicillin in lanolin, soft paraffin or benzoinated lard is not materially improved by the inclusion of formalin or buffering salts in the solutions. The following is the formula of an ointment containing a synthetic wax (polyethylene glycol ester). Formalin-treated, buffered penicillin solution, 10 ml. (1,000 Units), liphax 49, 10 g., sterilised liquid paraffin, up to 100 ml. This preparation has been found to retain 90 per cent. of its potency for at least 2 months. G. B.

Suppositories, Calculation of Quantities for Preparation of, by Cold Compression. V. G. Jensen and E. Jørgensen. (*Dansk Tidsskr. Farm.*, 1950, **24,** 9.) If it is necessary to prepare N suppositories of volume *a* ml., and assuming the average weight of suppositories made with the base only is A g., then a provisional mass (total weight C g.) is made containing the required amount of prescribed substance and less than the full amount of base, and the average weight of suppositories made from this is determined (B g.). The amount of base which it is necessary to add to the total mass is then given by the formula $\frac{(N \times B - C) A.}{B}$ Of the 2247 suppositories which were made in the course of this investigation, only 11 deviated by more than 5 per cent. from the required dose. Of these 9 were of chlorbutol and caffeine, which is always difficult. The maximum error in weight of suppositories prepared according to the above calculation was 3·4 per cent., and was in all cases considerably less than when an empirical calculation was used. G. M.

NOTES AND FORMULARIES

Doxylamine Succinate. (*New and Nonofficial Remedies. J. Amer. med. Ass.*, 1950, **142,** 33.) Doxylamine (decapryn) succinate, 2[α-(2-dimethylaminoethoxy)-α-methylbenzyl] pyridine succinate is a cream to white powder, with a characteristic odour. It is very soluble in water, freely soluble in alcohol and in chloroform and slightly soluble in benzene. The free base is obtained as an oil on the addition of sodium hydroxide solution. The melting-range is 100° to 104°C., and the *p*H of a 1 per cent. aqueous solution is 4·9 to 5·1. A 1 per cent. aqueous solution gives, with picric acid solution, a yellow gummy precipitate and with ammonium reineckate, a pink precipitate. 50 mg. mixed with 1 ml. of sulphuric acid gives a clear yellow to light orange solution which persists on standing. Succinic acid (melting-range 188°

to 190°C.) may be obtained from a solution by addition to ammonia, shaking out the base with ether and acidifying. Standards : $E_{1\,cm.}^{1\,per\,cent.}$ 260 mμ, 107 to 113; nitrogen by semi-micro Kjeldahl, 7·00 to 7·30 per cent., doxylamine succinate, by electrometric titration with alkali, 98·5 to 100·5 per cent.; limit of loss on drying *in vacuo* over phosphorus pentoxide for 5 hours, 0·5 per cent., and sulphated ash limit, 0·1 per cent. Doxylamine succinate is a histamine-antagonising substance with a marked sedative effect. G. B.

PHARMACOGNOSY

Anthraquinones and Anthraquinone glycosides. H. Muhlemann. (*Pharm. Acta Helvet,* 1949, **24,** 343, 356.) Reduction products of the anthraquinone glycosides appear to be the active substances present in anthraquinone drugs, though so far anthranol glycosides in a pure state have been isolated only from senna. Certain anthraquinone glycosides were therefore reduced to the corresponding anthranols and their chemical, physical and physiological properties were investigated. Reduction was effected in aqueous sodium hydroxide solution at room temperature using catalytic hydrogen and palladium-charcoal. Anthranols were first formed and later converted into anthrones. These hydrogenated compounds were very readily oxidised in air and could be isolated only with the utmost difficulty. They would not crystallise except after oxidation to the anthraquinone form. These facts may explain why, apart from the sennosides, no anthranol glycosides have been prepared in a crystalline form from plant material. The purgative activity of these glycosides was not much stronger than that of the original anthraquinone glycosides. Details are given for the preparation of the following compounds, chrysazin-anthrone-galactoside, chrysazin-anthrone-cellobioside, chrysazin-anthranol-cellobioside-acetate, emodin-anthranol-glucoside-A-heptacetate, and 1 : 7-dihydroxy-5-methylanthranol-glucoside-hexacetate. As a preliminary to the synthesis of various anthraquinone compounds, the author was able to prepare iso-cochinillic acid (4-methyl-6-hydroxy-1 : 2 : 5-benzoltricarbonic acid) by a relatively simple condensation. Full details of the method are given. On the basis of this method various derivatives of anthrone and anthraquinone have been prepared, e.g., 1-hydroxy-3-methyl-9-anthrone, 1-hydroxy-3-ethyl-9-anthrone, 1-hydroxy-3-carboxy-9-anthrone, 1-hydroxy-3-methyl anthraquinone, 1 : 5-dihydroxy-3-methyl anthraquinone. J. W. F.

Hyoscyamus muticus, **Botanical and Phytochemical Studies of.** Z. F. Ahmed and I. R. Fahmy. (*J. Amer. pharm. Ass.,* 1949, **38,** 479.) The formation of alkaloid during the development of the plant has been studied by the use of Wagner's reagent which precipitates hyoscyamine iodide within the cell in the form of characteristic crystals, and by alkaloidal assays of different parts of the plant and at different stages of development. Alkaloids first appear in the aerial parts and do not appear in the roots till about the fortieth day of growth. The highest alkaloidal percentage in the mature plant occurs in the floral parts, then the leaves, the stems and finally the roots which contain the lowest percentage. The best yield of alkaloid is obtained from the overground portions, in the flowering stage. The appearance of anthocyanin pigments in certain tissues seems to be followed by increased alkaloidal content. J. W. F.

Hysocyamus muticus, **Effect of Environment on the Growth and Alkaloidal Content.** Z. F. Ahmed and I. R. Fahmy. (*J. Amer. pharm.*

Ass., Sci. Ed., 1949, **38**, 484.) In order to produce the highest vegetative and alkaloidal yields, the following conditions of growth should be observed. Loamy soil with moderate irrigation, as excessive irrigation is detrimental. Application of nitrogenous fertilisers, especially inorganic. Ample sunshine which promotes the formation of hairs, calcium oxalate and colouring matter as well as alkaloids. Spring or summer sowing of the seeds. The plant is liable to attack by the root-knot nematode (*Heterodera marioni*); such infestation leads to low alkaloidal yields. J. W. F.

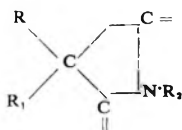
PHARMACOLOGY AND THERAPEUTICS

Bis-3:3′-(4-oxycoumarinyl) ethyl acetate (B.O.E.A.). A New Coumarin Substance. C. C. Burt, H. P. Wright and M. Kubik. (*Brit. med. J.*, 1949, 2, 1250.) In anticoagulant action, bis-3 : 3′-(oxycoumarinyl) ethyl acetate, has about one-fourth the activity, weight for weight, of dicoumarol. After a single dose, the minimal prothrombin level was reached between 8 and 24 hours and equally rapidly returned towards normal. With dicoumarol, the minimal level was not reached for 32 hours and was maintained for a comparatively long period. Clinical tests on 126 subjects treated therapeutically led to the conclusion that this compound is a step forward towards the production of an ideal anticoagulant, but prothrombin estimations must be carried out daily before the desired level is reached and at least every other day while the patient is under treatment. The cases included post-operative thrombosis and pulmonary embolism, spontaneous venous thrombosis, puerperal thrombosis, arterial thrombosis or embolism. In the majority of cases, 0·9 to 1·2 g. was given on the first 2 days, and thereafter dosage was regulated by response to treatment, 0·3 to 0·6 g. usually being sufficient. In over 80 per cent. of the patients the prothrombin level of the blood fell to under 50 per cent. of normal within 36 hours after the first dose and returned to over 50 per cent. of normal within the same period. The majority of patients were under treatment for 5 to 14 days, but in some cases treatment was continual for 3 to 4 weeks, 2 months and 10 months respectively. G. R. B.

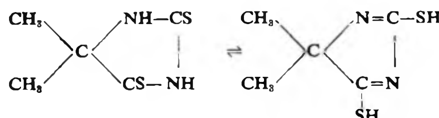
Decamethonium Iodide and Bromide; Effects on Neuromuscular Function and Induced Convulsions in Man. D. Grob, D. A. Holaday and A. M. Harvey. (*New Engl. J. Med.*, 1949, **241**, 812.) Intravenous administration of 2·5 to 3 mg. of C10 diiodide or of 2 to 2·75 mg. of C10 dibromide over a period of 5 seconds to 2 minutes produced complete relaxation of the muscles of the neck, arms, legs, shoulder girdle and pelvic girdle, and the degree of paralysis produced by this dosage could be maintained at a uniform level by further injections of 0·5 mg. every 4 or 5 minutes or 1 mg. every 8 or 10 minutes. If electric shock therapy was administered after C 10 at the height of the resulting paralysis the severity of both the tonic and clonic phases of the convulsion was lessened by as much as 50 per cent., without any apparent diminution of the therapeutic effect, and with no prominent subjective or objective effects except on neuromuscular function. The advantages of C 10 for the production of muscular relaxation prior to electrically produced convulsions, as compared with *d*-tubocurarine are shorter duration of action, and more rapid recovery of motor power, less pronounced effects on the pharyngeal, laryngeal, facial, and, in some cases, respiratory muscles, and the absence of any histamine-like effects. In the treatment of overdosage the intravenous injection of pentamethonium iodide

(C 5) did not result in a more rapid return of strength than occurred spontaneously, but the production of a high local concentration by the intra-arterial administration of 5 mg. did result in a slightly more rapid return of strength in the injected extremity. Neostigmine was ineffective. S. L. W.

Dimethyldithiohydantoin, a New Antiepileptic. R. Hazard, J. Cheymol, P. Chabrier and K. Smarzewska. (*Ann. pharm. franc.*, 1949, **7**, 569.) An examination of 40 compounds of the type depicted



showed that the maximum of anti-convulsive efficiency towards toxic crises, with the minimum toxicity, was obtained with dimethyldithiohydantoin. A new method of synthesis of this compound consists in treating a solution of 1 molecule of α-hydroxybutyronitrile in benzene with 2 molecules of carbon disulphide and 1 molecule of ammonia, dissolved in alcohol. After 24 hours, the mixture is filtered, the solution is evaporated, and the residue is taken up in 10 per cent. sodium hydroxide solution. The compound is then precipitated by the addition of sulphuric acid. After recrystallisation from benzene or hot water, it has m.pt. 142°C. It reacts in two forms:—



The calcium salt, which appears to be most suitable for clinical use, is prepared by treating the compound with excess of lime, in presence of water. After filtering, the solution is evaporated to dryness, and the residue is extracted with alcohol to remove excess of lime. This salt is stable (in the absence of carbon dioxide), but aqueous solutions decompose slowly in the cold, more rapidly on heating. Tests of toxicity, with mice and rabbits, indicate that the toxic dose for man is much greater than the useful therapeutic dose. The hypnotic dose (for mice) is 3 times that for phenobarbital. Therapeutic trials have been carried out on 190 epileptic hospital cases, and it is claimed that the compound offers the following advantages. Against phenobarbital: absence of hypnotic action in anti-convulsant doses. Against phenytoin: absence of vertigo, ataxia, nausea, etc. It therefore permits of ambulatory treatment. A dose of 1 g. is of value for facial neuralgia. A possible action on the thyroid has been noted; any considerable reduction in basal metabolism necessitates cessation of treatment, or the use of thyroid medication. G. M.

Procaine Penicillin and Sulphonamide Antagonism. H. Fischbach, H. Welch, E. Q. King, J. Levine, C. W. Price and W. A. Randall. (*J. Amer. pharm. Ass.*, 1949, **38**, 544.) The breakdown of procaine, derived from procaine penicillin, in the body to *p*-aminobenzoic acid and its possible antagonism to sulphonamide drugs has been studied. 36 male adults were injected intramuscularly with 1·5 million units of procaine penicillin (aqueous) representing 0·62 g. of procaine; 3 hours later, samples of blood were collected and immediately analysed for their penicillin content and for procaine or its derivatives. A new method was developed for the estimation of procaine and its metabolic products, a separation into three groups

being made by extraction with immiscible solvents at different pH values. Those fractions containing p-aminobenzoic acid derivatives are hydrolysed to liberate free p-aminobenzoic acid which is determined by diazotization and coupling with N(1-naphthyl)ethylenediamine to form a highly coloured derivative. Concentrations of less than 0·1 μg./ml. of p-aminobenzoic acid may be detected by this method. Results indicated that the greater proportion of the procaine is converted to N-acetyl-p-aminobenzoic acid. A small amount is converted to N-acetyl procaine, while in 7 out of 36 cases very low concentrations of free p-aminobenzoic acid were found; 6 cases showed a concentration of 0·1 μg./ml. or less and the remaining one 0·3 μg./ml. The molar ratios of various sulphonamides required to overcome the antagonistic action of p-aminobenzoic acid were: sulphanilamide 4000-1, sulphaguanidine 4000-1, sulphacetamide 500-1, sulphapyridine 400-1, sulphadiazine less than 100-1, and sulphathiazole 50-1; thus only 0·02 μg. of p-aminobenzoic acid per ml. of blood would neutralise 100 μg. per ml. sulphanilamide. The experimental findings coupled with the above ratios indicate therefore that procaine penicillin may be safely administered with sulphadiazine, sulphathiazole, and sulphapyridine, but may be antagonistic towards sulphanilamide.

R. E. S.

Streptomycin in Treatment of Tuberculosis and Other Infections. S. Kallner. (*Acta med. scand.*, 1949, **134**, 146.) Results obtained with streptomycin in about 50 cases are described. The cases treated were of 2 types, namely, acute tuberculosis, and infections proving resistant to sulphonamide and penicillin treatment. In fresh tuberculosis of the lungs with high fever a rapid effect was achieved, resembling the effect achieved with sulphonamide or penicillin treatment of acute infection. There was a rapid drop in temperature which was accompanied by detoxication. The patients felt better and their appetites and general health improved. In acute pulmonary tuberculosis the process was suspended so that a pneumothorax could be produced or thoracoplasty performed. Tuberculosis of glands, tuberculous osteitis and tuberculous fistulæ were successfully treated. Good results were frequently obtained with streptomycin (often where sulphonamide and penicillin had proved ineffective) in pyelitis and cystopyelitis, ulcerative colitis, chronic respiratory tract infections, especially when associated with bronchiectasis, and bronchial asthma complicated by purulent respiratory tract infection. S. L. W.

Thephorin Ointment in Pruritic Dermatoses. C. S. D'Avanzo. (*New Engl. J. Med.*, 1949, **241**, 741.) Thephorin (2-methyl-9-phenyltetrahydro-1-pyridindene), applied locally in the form of a 5 per cent. ointment in a carbowax vehicle was employed in the treatment of 74 cases of pruritic dermatoses. It was found of very little use in neurodermatitis disseminata; indeed, many of the patients became definitely worse. On the other hand, it was found of distinct therapeutic value in lichen chronicus simplex (neurodermatitis circumscripta) and chronic dermatoses associated with pruritus, approximately 50 per cent. being improved. About half the patients with initial improvement became refractory to continued use of the ointment. Four of the patients developed sensitisation to the ointment. S. L. W.

Thiourea Compared with Propylthiouracil in Thyrotoxicosis. G. T. Kent, R. A. Shipley and K. D. Rundell. (*Amer. J. med. Sci.*, 1949, **217**, 627.) The early decline in popularity of thiourea as an anti-thyroid drug was due

to its supposed low potency as indicated by animal assay and its high incidence of side-reactions in the dosage originally employed (1 to 2 g. daily). The object of the clinical investigation reported was to compare the usefulness of thiourea in small doses with that of propylthiouracil. Iodine (15 m. of Lugol's solution daily) was given with the thiourea in almost all cases and in a minority of cases with propylthiouracil. Both thiourea and propylthiouracil gave effective control in doses as low as 0·1 g. daily, though a daily dose of 0·2 or 0·3 g. daily of either drug gave more rapid improvement. Of 49 cases treated with thiourea, side-reactions, chiefly fever, occurred in 16 per cent.; no dangerous toxicity was encountered. In 51 cases treated with propylthiouracil there was one instance of fever, one of mild leukopenia and one of nausea, complete control of thyrotoxicosis occasionally required doses in excess of 0·3 g. daily. Iodine did not interfere with the efficacy of the drugs.

S. L. W.

Tolserol in the Treatment of children with Cerebral Palsy. E. Denhoff, R. H. Holden and C. M. Silver. (*New Engl. J. Med.*, 1949, **241**, 695.) Sixteen children, with ages ranging from 3 to 8 years, suffering from cerebral palsy, were treated with tolserol (3-*o*-toloxy-1:2-propanediol) for two 3-week periods. The average dose was 33 mg./lb. of bodyweight per 24 hours. The tolserol was administered in the form of a mixture with aqueous propylene glycol and syrup of cherry, so that 30 ml. of the mixture contained 1 g. of the drug. The mixture was administered to each child 6 times a day. There was no outstanding general improvement as a result of the treatment, but there was evidence that the drug caused a diminution of exaggerated reflexes and some improvement in co-ordination. Improvement was primarily in the neurologic category: 56·2 per cent. of the children showed diminution of exaggerated reflexes, and 12 per cent. became worse. There were no observable toxic effects, although for the first few days a few children complained of nausea and abdominal pain. S. L. W.

***d*-Tubocurarine Iodide, Dimethyl Ether of, as an Adjunct to Anæsthesia.** V. K. Stoelting, J. P. Graf and Z. Vieira. (*Proc. Soc. exp. Biol. N.Y.*, 1949, **69**, 565.) The dimethyl ether of *d*-tubocurarine iodide ($C_{40}H_{48}O_6N_2I_2,3H_2O$), referred to as M-curare, was administered to 100 anæsthetised patients of both sexes, ranging in age from 10 to 84 years. The drug was dissolved in distilled water and the curare adjusted to 0·5 mg./ml., the *p*H varying from 4·0 to 5·0. Injections were given intravenously, the initial dose consisting of 1 mg. or more of the drug. An average of 2 mg. of M-curare produced satisfactory relaxation in patients receiving cyclopropane anæsthesia; an average of 2·25 mg. was required with ether anæsthesia, and 3 mg. with nitrous oxide anæsthesia. The dosage was given in one or more injections within a period of 10 minutes. Adequate relaxation was not noted in any patients receiving less than 1 mg. of M-curare. Relaxation produced by the initial dose sufficed for surgical procedures lasting for 60 to 90 minutes; after this, supplementary injections of 0·5 to 1 mg. were infrequently required. No cardiovascular changes were noted in any of the patients and mild respiratory depression was observed in 9 out of the 100 patients, in 7 of which it existed before surgery. The relaxation obtained with M-curare in this study was comparable with that obtained with *d*-tubocurarine chloride. The drug appears to have a selective action on skeletal muscle similar to that of *d*-tubocurarine chloride, but seldom affects the muscles of respiration. Less M-curare than *d*-tubocurarine chloride is needed on a weight for weight basis. S. L. W.

BOOK REVIEWS

MARIHUANA IN LATIN AMERICA, by P. O. Wolff. Pp. 56. Linacre Press Inc., Washington 6, D.C., U.S.A. 1949. $1.50.

The main purpose of this pamphlet is to examine the medical, legal and criminal aspects of the problems raised by the growth of addiction to Indian hemp. The use of the drug in the East has been recorded from the earliest times, but it has spread to the United States, Mexico, Brazil and certain other parts of Central and South America to such an extent that the total number of addicts throughout the world is estimated at 200 millions. In the United States and Spanish-speaking America the drug is known as marihuana, and in Brazil as maconha and under innumerable other local names. Unlike opium and its derivatives, marihuana has no medicinal value to justify its production and use, but addiction has been facilitated by the ease of cultivation of the plant, the simplicity of preparation of the drug and the low price. Those engaged in combating the menace of cannabis in Latin America will be greatly indebted to the author for this review of the problems. As Secretary of the Expert Committee on Habit-forming Drugs of the World Health Organisation, Dr. Wolff has brought to the work his wide knowledge and experience of the subject. T. C. DENSTON

BENNETT'S MATERIA MEDICA AND PHARMACY FOR MEDICAL STUDENTS, revised by H. G. Rolfe. 5th Edition. Pp. xxviii + 276. H. K. Lewis and Co., Ltd., London. 16s.

The excellent qualities of Mr. Bennett's book which have brought it to the 5th edition are continued in the new issue and the purpose of presenting to the medical student a concise account of the drugs, chemicals and preparations of the British Pharmacopœia is well fulfilled. Within this field it gives the descriptive data about pharmaceutical preparations which are generally omitted from textbooks of pharmacology. In limiting the preparations discussed to those of the pharmacopœia, however, the author has had to omit numerous drugs which are now of considerable importance. Among these are sulphadimidine, phthalylsulphathiazole, butobarbitone, amylobarbitone and papaveretum. In a future edition it might be advisable to include some of the more important preparations of the British Pharmaceutical Codex. The introduction, describing various pharmaceutical preparations, is very useful and could with advantage be extended. The descriptions of drugs and preparations are mostly compact and accurate and the brief notes on actions and uses should be of considerable revision value to the medical student. The strict adherence to official nomenclature in the text is commendable, but the inclusion of a glossary of synonyms and trade names would increase the value of the book to medical students. The text is remarkably free from typographical errors. T. D. WHITTET

PHARMACOPŒIAS AND FORMULARIES

CODEX MEDICAMENTORUM NEDERLANDICUS

The Dutch Codex stands in a somewhat similar relation to the Pharmacopœia of the Netherlands as the British Pharmaceutical Codex does to the British Pharmacopœia. This, the second edition, is published in two volumes. The first contains introductory matter and monographs on chemical and vegetable substances, galenical preparations, a few homoeopathic remedies and materials such as catgut. The second volume resembles in some respects the Formulary section of the British Pharmaceutical Codex, with the addition of a number of prescriptions. Division of the work into two volumes is no doubt a convenience to those whose interests are restricted to, or chiefly concerned with, one part only. It may, however, introduce a tendency for readers to overlook general considerations or specific requirements for an ingredient, dealt with in the first volume, when referring to the formula of a preparation given in the second volume. The recommendations on nomenclature published in the Interim Report on the Unification of Pharmacopœias have not been adopted completely, notably with vegetable drugs which appear under titles such as Radix Belladonnæ, not Belladonna Radix. Similarly the style of the monographs differs from that used in English and American counterparts by the omission of side headings printed in bold-faced type, which afford useful guidance when consulting particular points.

The monographs cover a wide range, from modern synthetic drugs to vegetable substances of antiquity as, for example, olibanum and manna. There are specifications for six barbiturates, including cyclobarbitone and methylphenobarbitone, for vitamins such as aneurine hydrochloride and nicotinamide, and for ergometrine and amphetamine. Proprietary names are given at the foot of the monographs, a practice which involves no fewer than 14 brand names for Opium Concentratum (Papaveretum). The requirements for trichloroethylene are not as comprehensive as might be expected for an anæsthetic, particularly in the provision of limit tests for likely impurities.

Vegetable substances are represented by monographs on 25 herbs and leaves, 7 flowers, 14 fruits and seeds, 9 barks, and 13 rhizomes and roots. The drugs range from senna fruit and belladonna root to those of less significance in medical practice in this country, such as calendula flowers, matico leaf, shepherds' purse and fresh sundew. Microscopical characters are described in detail, with cross-reference to a series of 60 drawings printed at the end of this volume as illustrations of the diagnostic features. Scammony resin is attributed to scammony root derived from *Convolvulus Scammonia* Linn., a source which does not appear to have been available in this country for many years.

The second volume contains, as Part IIA, nearly 300 formulæ including a wide selection of injections and tablets. As in Part I, brand names are given and, indeed, some formulæ appear to be imitations of patent medicines well-known in Britain. Preparations for which formulæ are not usually provided in books of this character published in English include tooth-paste, tooth-powders and denture powders. Part IIB consists of a selection of prescriptions such as may be found in a modern prescribers' formulary and others less frequently encountered nowadays, such as Pilulæ Cochiæ and Mistura Ferri Composita (Griffith's Misture). The volume concludes with sections comprising the Formularium Medicamentorum Nederlandicum and the Formularium Medicamentorum Indicum.

T. C. Denston.

LETTER TO THE EDITOR

Adrenaline and Blood Potassium

It is well known that the intravenous injection of adrenaline into mammals results in a transient, though very marked and prompt, increase in the serum potassium. By using adrenaline-like substances, D'Silva[1] showed that the reaction appeared to depend on the catechol nucleus and on the presence of a hydrogen atom linked directly with nitrogen on the side-chain. When the portal area was excluded from the circulation, none of these substances increased the serum potassium 1 minute after injection. We have now obtained results in a series of cats under chloralose anæsthesia to show that the rise in serum potassium following the intravenous injection of adrenaline can be greatly reduced, or even prevented, by the previous administration of dibenamine, an anti-adrenaline agent. A typical example of this action is shown in Table I, estimations of the potassium content of the serum being carried out by the colorimetric method of Abdul-Fadl.[2]

TABLE I

Effect of dibenamine (15 mg./kg) on the rise of serum potassium produced by the intravenous administration of adrenaline (50 μg.)

	Serum Potassium (mg./100 ml.)		Difference calculated as a percentage over the control value
	Before Adrenaline	After Adrenaline	
Before Dibenamine	17·3	38·0	+120
After Dibenamine	21·3	20·0	− 5

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References

1. D'Silva, J. L., *J. Physiol.*, 1949, **108,** 218.
2. Abdul-Fadl, M. A. M., *Biochem. J.*, 1949, **44,** 282.