# REVIEW ARTICLE

## LA STÉRILISATION DES SOLUTIONS INJECTABLES

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LA préparation de liquides injectables stériles ne semblait pas poser, il y a une trentaine d'années, de problèmes scientifiques très difficiles. Mais à mesure que l'emploi en thérapeutique de substances chimiques de plus en plus fragiles s'est généralisé, il a fallu renoncer à employer dans tous les cas des méthodes de stérilisation par la chaleur considérées comme sûres. Il n'est donc pas sans intérêt de jeter un coup d'œil général sur l'évolution historique des interprétations théoriques de la stérilisation, et sur les modifications des techniques pratiques qui en ont résulté dans les quatre-vingts dernières années. Nous verrons ensuite quelles sont les positions officielles adoptées par les dernières Pharmacopées britannique, américaine et française, et comment la notion de stérilité des solutés injectables se présente à l'heure actuelle.

### I. THÉORIES GÉNÉRALES DE LA STÉRILISATION ET APPLICATION AU CAS DES SOLUTÉS.

1. *La chaleur.*—Les premiers chercheurs dans ce domaine avaient en vue, à la fois, un problème théorique très général: celui de l'origine de la vie, et un problème pratique: celui de la conservation des liquides riches en matières organiques, tels que les sucs de plantes (obtenus par pression de la plante fraîche), ou les infusions de plantes sèches. C'est au XVIII[ème] siècle qu'ont été faites les découvertes fondamentales, en particulier celle de Spallanzani[1], qui établit le premier, d'une manière indiscutable, qu'une infusion végétale était rapidement envahie par de petits organismes vivants quand elle n'avait pas été chauffée, tandis que les êtres vivants n'apparaissaient pas quand l'ensemble du vase scellé contenant l'infusion avait été porté à la température de l'ébullition. Plus tard, le français Appert[2] généralisait cette conclusion en mettant au point un procédé de préparation de conserves alimentaires stérilisées par chauffage au voisinage de 100°C. Mais c'est seulement Pasteur qui a élucidé d'une manière complète le mécanisme de ce phénomène, au cours de ses recherches célèbres sur l'inexistence de la génération spontanée. Pasteur[3] a établi en effet plusieurs faits très importants: 1°) Un liquide organique porté à une température suffisante en vase clos, demeure ensuite indéfiniment limpide, aucun germe vivant ne se développant dans le milieu. 2°) S'il est mis en contact, après chauffage, avec de l'air, il y a contamination par des germes vivants existant en suspension dans l'atmosphère, mais cette contamination est évitée à la condition d'établir la communication avec l'air par l'intermédiaire d'un tube capillaire très long et recourbé. Ce n'est pas le contact de l'air qui provoque la contamination, mais le contact de germes en suspension dans l'air.

Une autre notion fondamentale est celle de la distinction entre les conditions de destruction par la chaleur des formes végétatives des bactéries (rapidement détruites au voisinage de 60°C.) et les spores bactériennes beaucoup plus résistantes, détruites seulement par un chauffage à l'autoclave aux environs de 120°C. C'est Tyndall[4] qui a constaté l'insuffisance du chauffage à l'ébullition, même prolongé, vis-à-vis de certaines spores. Il a également eu le mérite de montrer qu'on disposait d'un autre moyen pour les détruire. On peut en effet: ou bien maintenir pendant plusieurs heures la température aux environs de 100°C.—et la destruction peut alors être effectuée en une seule opération —ou bien effectuer cinq chauffages à 100°C. d'une minute par exemple chacun, avec des intervalles de 24 heures entre deux chauffages consécutifs, et l'effet obtenu est sensiblement le même.

Cette méthode de chauffage discontinu, qui a reçu le nom de " tyndallisation," pose l'un des problèmes les plus surprenants de la biologie. En effet, l'explication qu'avait donnée Tyndall lui-même de l'efficacité de la méthode—explication qui est devenue tout de suite classique et qui continue d'être admise par de nombreux auteurs, même aujourd'hui— était la suivante: Dans l'intervalle de deux chauffages, la spore bactérienne donnerait naissance à une forme végétative et deviendrait, à ce moment, vulnérable. Elle pourrait donc être facilement détruite lors d'un chauffage ultérieur. En répétant plusieurs fois l'opération, on finirait par détruire, au moment où elles prennent la forme végétative, toutes les bactéries issues de spores. C'est Duclaux[5] qui a mis en doute cette interprétation. Il a, en effet, maintenu *à la glacière* le liquide, dans l'intervalle de chauffages consécutifs. Il est évident que, dans ces conditions, aucun développement bactérien ne peut avoir lieu, et que les spores ne peuvent donner naissance à des formes végétatives. Cependant, d'après Duclaux, la tyndallisation reste efficace même dans ce cas. On serait donc conduit à admettre, comme l'a fait Duclaux, que c'est bien la *discontinuité* du chauffage qui, par un méchanisms entièrement inconnu, permet la destruction de la spore.

Les techniques de la stérilisation par la chaleur se sont précisées entre 1880 et 1900, et elles découlent directement des travaux précédents. 1°) Stérilisation par chauffage à l'autoclave après purge d'air pendant un temps qui dépend de la nature du liquide et de la température adoptée (en général: 110°, 115° ou 120°C.). Nous reviendrons plus loin sur ce point. 2°) Tyndallisation en trois opérations de 10 minutes à une heure chacune, avec 24 heures d'intervalle. La température adoptée dépend de la fragilité des substances existant en solution. La température minimum utilisable est de 56°C.; chaque fois qu'on le peut, on opère à 70° ou 80°C.

Précisons maintenant la relation entre durée de chauffage et température. Il est tout à fait important d'insister sur le fait qu'il n'existe pas une température critique mortelle caractérisant chaque espèce bactérienne. En réalité, le phénomène semble être soumis, au moins en première approximation, au calcul des probabilités, c'est-à-dire qu'à une certaine température, et dans un certain milieu chimique, une bactérie

déterminée, sous forme végétative, aurait une certaine *probabilité* d'être détruite, en un temps d'une seconde par exemple. Pour préciser les données numériques, on admettra donc que la destruction est totale quand il ne reste plus, en moyenne, qu'une bactérie par litre (ou par mètre cube) par exemple. La vérification de cette proportion moyenne de survie après stérilisation est en réalité *impossible*, et on admet couramment que la stérilisation est complète quand on a chauffé, par exemple 5 minutes à 60°C., une émulsion de *B. typhosum*[6], tandis que pour le bacille thermophile, il faudrait envisager un chauffage de même durée à 150°C. Enfin, pour ce dernier germe, la variation du temps de chauffage nécessaire avec la température suit une loi différente[7], comme

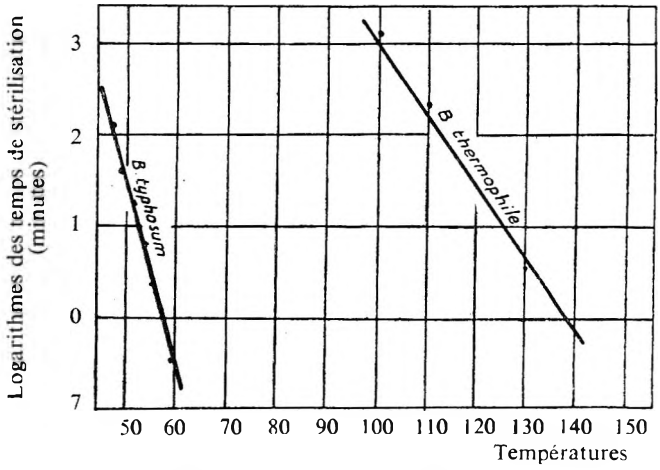


FIG. 1.—Relation entre la durée de stérilisation à l'autoclave et la température.

l'indique la Fig. 1, sur laquelle on voit que le temps de chauffage doit varier beaucoup plus quand on passe de 60° à 50°C. pour *B. typhosum*, que quand on passe de 140° à 130°C. pour le bacille thermophile. On a d'ailleurs trop souvent tendance à penser qu'une diminution de 10°C. dans la température de chauffage pourra être compensée par une légère augmentation de durée de stérilisation, alors qu'en réalité la durée doit être multipliée par 6 ou 7 aux environs de 115°C. pour des bactéries sporulées.

2. *Les rayonnements.*—Dans les plus anciens des travaux, on avait tendance à rechercher quelle était, pour une certaine culture et une certaine source de rayonnement, la durée d'irradiation qui amenait la mort de tous les organismes de la culture. On parlait donc de " dose léthale " ou de " dose de stérilisation." Quand on a pu travailler dans des conditions rigoureuses, c'est-à-dire avec une source d'intensité constante fournissant un rayonnement monochromatique de longueur d'onde connue, et avec une culture d'organismes identiques les uns aux autres, en nombre connu, on a vu qu'en réalité, la mortalité de ces organismes était affaire de probabilité et qu'à chaque durée d'irradiation

correspondait une certaine probabilité de survie qui devenait très petite quand la durée devenait grande, mais qui n'était jamais nulle. Plus tard, quand la théorie des quanta s'est trouvée bien établie, on a admis que le rayonnement *X* utilisé ne pouvait être émis ou absorbé par n'importe quelle *molécule* chimique, que par quantités toujours égales, caractéristiques du rayonnement considéré. Chacun de ces *quanta* d'énergie électro-magnétique ondulatoire a pour valeur $\frac{hc}{\lambda}$ (h, constante universelle de Planck; c, vitesse de la lumière; $\lambda$, longueur d'onde du rayonnement considéré). On s'est alors posé la question de savoir si les *organismes vivants* n'absorbaient pas, eux aussi, le rayonnement *X* par nombres entiers de quanta[8]. La démonstration a été faite par Holweck et Lacassagne[9], sur des cultures de *Saccharomyces*. Ils ont comparé les courbes de mortalité obtenues, avec les différentes courbes calculées, en supposant que chaque levure était tuée quand elle avait absorbé soit 1, soit 2, soit 3, etc., soit *n* quanta (Fig. 2). Et ces auteurs ont trouvé que

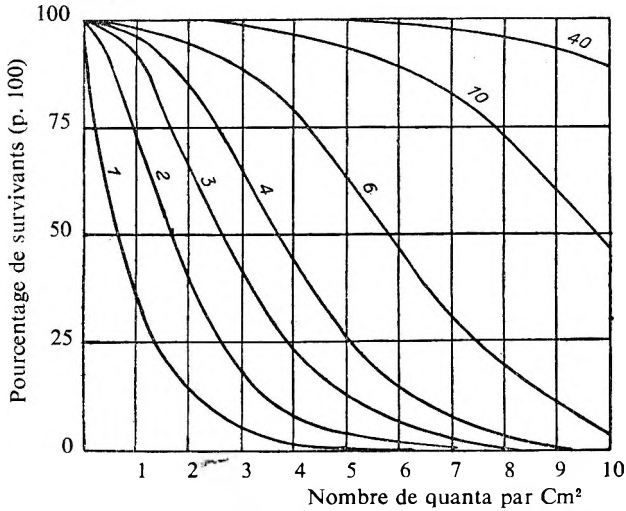


FIG. 2.—Courbes de survie calculées par F. Holweck. (Pourcentage de survivants en fonction du nombre de quanta de rayonnement irradiant 1 cm² de culture.)

l'allure des courbes était bien celle que faisait prévoir le calcul et qu'on pouvait, rien que d'après la forme de la courbe expérimentale, dire combien de quanta étaient nécessaires pour tuer chaque individu. Sans entrer dans le détail du mode d'action de ces photons sur la matière vivante, disons que des expériences analogues ont été faites avec des rayonnements *X* de longueur d'onde plus grande (rayons *X* mous), puis avec des rayons ultra-violets. A mesure que la longueur d'onde augmentait, des rayons *X* durs aux ultra-violets (ou, ce qui revient au même, à mesure que l'énergie du quantum diminuait), le nombre de ces quanta nécessaires pour tuer un organisme augmentait aussi. Egal à trois ou quatre pour des rayons *X* moyens, ce nombre atteint trente à quarante pour les rayons ultra-violets. Ce qui se traduit, quand on

regarde la famille des courbes de survie calculées mathématiquement, par le passage d'une courbe très rapidement décroissante, dès son départ, à une courbe qui présente un palier initial considérable. Si les deux courbes sont ramenées à la même échelle relative, il est facile de les distinguer l'une de l'autre au premier coup d'œil (Fig. 3). Dans le cas de la courbe des rayons *X*, dès le début de l'irradiation un assez grand nombre d'organismes sont tués. Au contraire, dans le cas de la courbe des rayons ultra-violets, au début de l'irradiation aucun organisme ne semble d'abord touché et il continue d'en être ainsi jusqu'au moment où, brusquement, sans que les conditions d'irradiation aient été modifiées, une mortalité considérable, et comme contagieuse, apparaît. Cela tient à ce que, dans le premier cas, pour que trois quanta soient venus frapper une même cellule de levure, il ne fallait pas un temps bien long. Tandis que, dans le deuxième cas, pour que la première cellule soit tuée, il faut qu'elle ait eu le temps d'être bombardée quarante fois. Pendant un certain laps de temps, la culture contient des cellules ayant reçu des nombres variables de quanta. Tant qu'aucune n'en a reçu quarante, toutes survivent. Mais dès que ce nombre est atteint pour une d'elles, il a de très grandes chances de l'être pour les autres, presque aussitôt. Et c'est pourquoi la chute de la courbe, après le palier, est brusque.

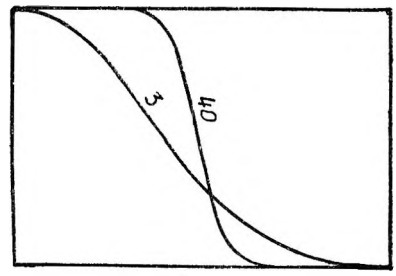


FIG. 3.—Comparaison des courbes de survie correspondant aux rayons ultra-violets (courbe 40) et aux rayons X (courbe 3) ramenés à la même échelle relative.

Les expériences faites en substituant la chaleur agissant par rayons infra-rouges (de longueur d'onde bien plus grande encore que celle des ultra-violets) n'ont pas donné de résultats aussi clairs. Cependant, des physiciens ont été tentés de penser que, puisque les quanta infra-rouges avaient une énergie encore plus petite que celle des quanta ultra-violets, il en faudrait beaucoup plus de quarante pour tuer un même organisme. On devait s'attendre à une courbe, à palier initial encore plus long, à chute très brusque. Cette notion, étendue au cas de la stérilisation des bactéries par la chaleur, paraît, à première vue, très séduisante: dès qu'on aura dépassé la dose correspondant à la fin du palier, on verra le pourcentage de survie tomber très rapidement, pour une légère augmentation de la durée de chauffage, à des valeurs infiniment faibles. Comme, d'autre part, les courbes de mortalité obtenues—au moins dans certains cas—par action d'antiseptiques sur des bactéries semblent se rapprocher plutôt des courbes des types 3 à 10, on est tenté de dire qu'on a compris pourquoi la stérilisation par la chaleur est meilleure que celles obtenues par les rayons *X*, les antiseptiques ou même les rayons ultra-violets: la *décroissance* de la probabilité de survie, au delà d'une dose minimum indispensable, serait plus rapide avec la chaleur qu'avec tous les autres modes de stérilisation.

Mais, l'examen des résultats expérimentaux contredit tout de suite ces hypothèses séduisantes :

Examinons, par exemple, les résultats d'une expérience de Eijkmann[10] faite sur une émulsion de *Bact. coli* dans le sérum physiologique, à la température de 52°C. (Fig. 4). Dès le début du chauffage, on voit le

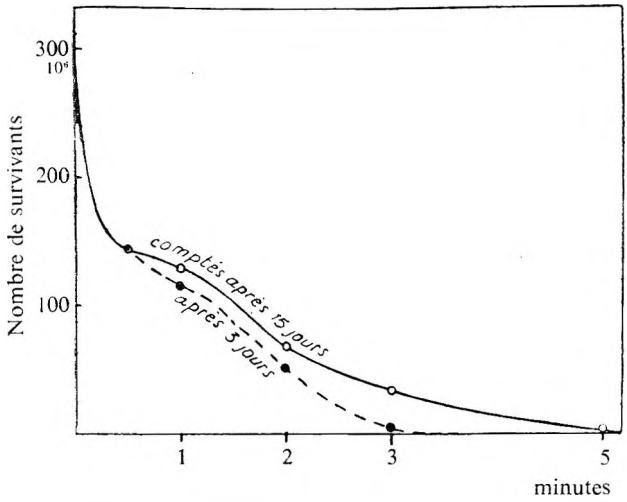


FIG. 4.—Influence de la durée de chauffage sur le nombre de *Bact. coli* survivants (d'après Eijkmann) (t=52°).

nombre des bactéries vivantes diminuer considérablement : 50 p. 100 sont tuées en moins d'une demi-minute. Et, pour que 50 p. 100 des survivants disparaissent encore, il faut continuer l'irradiation plus d'une minute. Ainsi, au lieu d'un palier initial, c'est le contraire qu'on observe : la décroissance de la courbe, *à son début*, est *plus rapide* même que celle d'une courbe exponentielle simple qui correspondrait à $n = 1$ (*un* quantum absorbé par bactérie). La théorie précédente est donc en défaut.

Enfin, un dernier fait expérimental important vient compliquer les expériences d'Eijkmann : il évalue d'abord les bactéries survivantes après trois jours d'incubation à 37°C. Mais si on prolonge cette incubation *quinze jours à 37°C.*, on voit de nouvelles colonies apparaître tardivement, auxquelles correspond une courbe qui présente la même allure que la précédente, mais se prolonge plus loin. Or, initialement, tous les germes de la culture se développaient à 37°C. en moins de trois jours. C'est le chauffage qui a transformé certains d'entre eux en germes à développement très lent. Et, de plus, ces germes sont les plus résistants à la chaleur, puisqu'ils subsistent seuls, à la fin de l'expérience, quand les autres sont déjà pratiquement tous tués. Il nous faut conclure non seulement que la population microbienne en expérience était initialement hétérogène, mais qu'une nouvelle hétérogénéité, à laquelle correspond une plus grande résistance à la chaleur, a été *provoquée* par le chauffage.

Ainsi, l'étude de l'action stérilisante des rayonnements sur les bactéries

a seulement permis de mieux comprendre la complexité de la stérilisation par la chaleur. On voit qu'avant que la stérilisation ne devienne une opération mathématiquement interprétable, de nombreux travaux restent à faire.

3. *Les antiseptiques.*—Ici encore, la notion de probabilité va intervenir. Pendant longtemps, on a pensé qu'à une certaine concentration, un antiseptique tuait *tous* les germes présents dans une solution, quels que soient leur nombre et leur nature. On sait maintenant que le processus est moins simple :

1) la résistance des germes dépend de *l'espèce* bactérienne et, de plus, n'est pas la même pour les formes végétatives et les spores;

2) le résultat obtenu est fonction du *nombre* de germes présents initialement par ml.;

3) il est fonction aussi de la *durée* de contact;

4) toutes les constantes physico-chimiques de la solution interviennent : *p*H, pression osmotique, potentiel d'oxydo-réduction, etc.

Enfin, après un contact prolongé avec des solutions peu concentrées, le germe peut devenir *résistant* à l'antiseptique, ce qui complique beaucoup les choses.

Un grand progrès a été accompli le jour où l'on a distingué le pouvoir antigénétique, ou *bactériostatique*, d'un antiseptique, qui est la propriété d'empêcher le *développement* des germes de la solution, et le pouvoir *antibiotique*, qui correspond à la *mort* des germes, et non pas seulement à l'arrêt de leur reproduction. En général, cette distinction correspond à des concentrations différentes de la même substance. Mais il peut arriver qu'une substance faiblement antiseptique ne possède qu'une action bactériostatique, même à concentration élevée. Dans le cas où un liquide aura subi l'une de ces deux actions, si, par dilution, on rend négligeable l'action de l'antiseptique, on verra la croissance des bactéries se déclencher s'il y a eu seulement action bactériostatique, tandis que la stérilisation restera efficace s'il y a eu action antibiotique. Comme dans le cas de la température, on aura à considérer une relation entre la concentration et le temps de contact nécessaire, pour une bactérie déterminée, et ces chiffres varieront considérablement d'une bactérie à l'autre; ils varieront plus encore dans leur ensemble, quand on passera d'un antiseptique à un autre. Si l'on suppose que l'action antiseptique est due à la rencontre d'une ou plusieurs molécules de la substance active avec la bactérie, l'interprétation mathématique du phénomène est tout à fait analogue à celle de l'interaction d'un certain nombre de quanta de rayonnement avec une bactérie. On retombe par conséquent sur le problème étudié par Holweck, et le comportement d'un antiseptique vis-à-vis d'une culture homogène d'un germe déterminé devra être représenté par une courbe de survie de forme caractéristique, dont la seule vue permettra de savoir quel est le nombre de molécules nécessaires, en moyenne, pour que l'action se manifeste. Toutefois, pour les raisons déjà exposées plus haut en ce qui concerne les rayonnements, des études expérimentales de ce genre sont très difficiles et n'ont été qu'ébauchées jusqu'ici.

Nous voyons donc que les mécanismes d'action d'agents stérilisants

aussi différents que la chaleur, les rayons *X* ou ultra-violets, et les antiseptiques, paraissent justiciables d'interprétations théoriques assez analogues, mais que les problèmes pratiques posés du fait de la multiplicité des germes possibles et de leur résistance variée, rendent une étude scientifique précise à peu près inextricable.

4. *Filtration.*—Un procédé de stérilisation plus élégant que tous les précédents, et qui a été reconnu efficace dès les origines de la bactériologie[11,12], est le procédé par simple filtration. Cependant, ici encore, les facteurs qui interviennent sont multiples et la conduite scientifique de l'opération est difficile. On sait depuis longtemps qu'un filtre poreux, dont les trous ont un diamètre plus grand que celui des bactéries, *peut* néanmoins les arrêter. On attribue ce fait à des actions électrostatiques, la paroi du filtre prenant une charge électrique par adsorption d'ions, et la bactérie elle-même pouvant posséder une charge inverse, du fait de la fixation irréversible d'un grand nombre d'ions sur les groupements carboxylés ou aminés des protéines constitutives. Cette fixation d'ions est elle-même conditionnée par toutes les constantes physico-chimiques du soluté.

En admettant même que cette filtration ait été parfaitement efficace, sa plus grande difficulté d'emploi reste la nécessité de recueillir *ensuite* le liquide filtré dans des conditions d'aseptie parfaite et de le répartir en ampoules qu'on devra enfin sceller. Les opérations industrielles de ce genre sont devenues possibles depuis quelques années, grâce à une technique qui a été mise au point, surtout aux Etats-Unis[13]:

a) Elimination de la presque totalité des bactéries existant en suspension dans l'air par un groupe de dépoussiérage industriel à trois étages:

1) Dépoussiérage électrique Cotrell.

2) Arrêt mécanique par un système de surfaces huilées disposées en chicane.

3) Arrêt par condensation d'un brouillard.

b) Emploi de lampes à rayons ultra-violets ayant un maximum d'intensité dans la bande de 1900 à 2000 Å, de manière à détruire toutes les bactéries subsistant dans l'air. Murs à pouvoir réfléchissant élevé.

c) Emploi par le personnel de masques chirurgicaux, lunettes protectrices contre le rayonnement ultra-violet, vêtements spéciaux stériles, bottes, etc. . . .

Dans ces conditions, la manipulation des liquides stérilisés peut être faite avec un minimum de chances de contamination. On peut encore, pour plus de sécurité, réaliser le plus d'opérations possible en vase clos, dans des appareils en verre entièrement stérilisables au préalable, mais quelles que soient les précautions prises, on n'élimine jamais *totalement* la présence possible de quelques rares bactéries, et il est nécessaire, pour neutraliser ce risque, d'ajouter à la solution une substance bactériostatique.

5. *Cas où on peut ne pas stériliser.*—Les liquides existant normalement dans l'organisme vivant sont presque toujours des solutés stériles. Il est donc possible d'en opérer le prélèvement dans des conditions aseptiques et d'obtenir *directement*, sans stérilisation, ni filtration, un soluté exempt

de bactéries. C'est ce qui se produit quand on recueille, avec toutes les précautions nécessaires, du sang humain, et qu'on sépare les éléments figurés du plasma. Ce plasma peut être théoriquement desséché dans le vide, à l'abri des contaminations, et mis en ampoules. Il suffira ensuite de redissoudre dans de l'eau stérile pour réobtenir un plasma injectable[14].

6. *Voies d'injection et stérilisation.*—Les chances d'infection par introduction d'un liquide incomplètement stérilisé, dans des points différents de l'organisme humain, sont très variables. Par voie sous-cutanée ou intra-musculaire, ainsi que par voie intra-rachidienne, on ne peut introduire que des liquides rigoureusement stériles ou additionnés d'un bactériostatique, sous peine de provoquer des infections. Par contre, on sait depuis longtemps que l'injection intra-veineuse d'un liquide contenant quelques bactéries est généralement sans inconvénient, à moins qu'il ne s'agisse de bactéries pathogènes, de sorte que la stérilité rigoureuse a *moins* d'importance dans ce cas que dans les autres. On sait, en particulier, que pendant la digestion, des germes bactériens apparaissent transitoirement dans la circulation sanguine.

## II. Evolution Historique des Techniques de Préparations des Solutés Injectables.

Lorsque les solutés injectables sont entrés dans la pratique thérapeutique après 1870, les travaux de Pasteur avaient déjà mis en lumière la notion de stérilisation. Néanmoins, on commença par se contenter de dissoudre les alcaloïdes dans des solutions acides qu'on utilisait sans stérilisation. C'est ainsi qu'Adrian[15], en 1872, prescrivait d'utiliser l'alcaloïde base, qu'il dissolvait dans l'eau distillée bouillie, additionnée de 20 p. 100 de glycérine, à l'aide d'acide sulfurique dilué au $1/10^{ème}$. Le soluté ainsi préparé n'était ni neutre, ni stérile, ni isotonique. Il se conservait néanmoins, à cause de son acidité, mais l'injection était évidemment douloureuse. Vers 1900, se généralisa la pratique d'une stérilisation sommaire par la chaleur: on dissout le médicament dans l'eau distillée bouillante et on filtre dans un flacon muni d'un bouchon de liège. On porte ensuite ce flacon bouché à la température de 100°C. pour stériliser sommairement la solution. Généralement, pour éviter une surpression, on plaçait une ficelle entre le bouchon et le goulot, ficelle qu'on enlevait après chauffage pour fermer définitivement le flacon.

En France, c'est le Codex de 1908[16] qui a, pour la première fois, précisé les conditions de préparations des solutés injectables stériles. Il donnait le choix entre le procédé qui vient d'être indiqué et l'emploi de l'autoclave à 110°C. pendant 10 minutes. Les solutés injectables figurant au Codex de 1908 étaient ceux de caféine et benzoate de soude, caféine et salicylate de sodium, chlorure de sodium, chlorure de sodium et sulfate de sodium, chlorhydrate de cocaïne, chlorhydrate de morphine et quinine-antipyrine.

Au Formulaire des Hôpitaux militaires français de 1909[17], il est prescrit de préparer les solutés injectables, soit dans des fioles à sérum munies d'un bouchon de caoutchouc, soit dans des ampoules scellées. Le mode de stérilisation adopté est le chauffage discontinu durant 20 minutes à

100°C. trois fois, à 24 heures d'intervalle. Dans le cas des solutés fragiles, le chauffage discontinu est effectué une heure à 58 ou 60°C., et répété cinq à six fois à 24 heures d'intervalle (biiodure de mercure, ergotine). La solution d'ergotinine devait être préparée aseptiquement et non stérilisée, en raison de la fragilité de l'alcaloïde. Seule, la solution isotonique de chlorure de sodium était stérilisée à l'autoclave (à 120°C. pendant 30 minutes).

Au Codex français de 1937, encore en vigueur, on adopte deux techniques de stérilisation[18]; soit le chauffage discontinu à 70°C. pour tous les solutés de médicaments altérables (alcaloïdes, salicylate de sodium, novocaïne-adrénaline, etc.), soit stérilisation à l'autoclave à 110°C. pendant 20 minutes pour tous les solutés peu fragiles. Le soluté d'apomorphine est exceptionellement stérilisé 30 minutes à 100°C. et la solution injectable de gélatine, deux fois 15 minutes à l'autoclave à 115°C. (en raison de la possibilité d'existence de spores de tétanos dans la gélatine). La filtration à la bougie n'est préconisée que pour la stérilisation des solutés injectables opothérapiques, mais le Codex recommande de tyndalliser après remplissage des ampoules.

En Angleterre[19] les injections hypodermiques ont fait leur apparition à la Pharmacopée britannique de 1885. On se contentait de dissoudre à douce chaleur, et de filtrer, en employant l'eau camphrée, comme solvant, dans le cas de l'apomorphine et de l'ergotine, et de l'eau additionnée d'ammoniaque et d'acide acétique dans le cas du chlorhydrate de morphine. Au Codex britannique de 1934, figurent des solutés injectables de digitaline et de thiosinamine-salicylate de soude, qui sont stérilisés, soit par tyndallisation, soit par filtration, et des solutés de morphine, strychnine, quinine-uréthane, morrhuate de sodium et peptone qui sont stérilisables au choix, soit à l'autoclave, soit par tyndallisation, soit par filtration.[20] Ce codex ne manifestait donc pas de préférence nette pour un procédé plutôt que l'autre. C'est déjà ce que faisait la Pharmacopée britannique de 1932, qui laissait le choix généralement entre plusieurs des trois mêmes méthodes. La Pharmacopée britannique de 1932 indique la manière de préparer d'urgence des solutés injectables[21]: la solution doit être additionnée d'un bactériostatique, à une dose équivalant à 0·5 pour cent de phénol; elle est ensuite mise en récipient stérile, et stérilisée par chauffage à 80°C. pendant 30 minutes, une seule fois. L'étiquette doit porter l'indication " A mettre en lieu frais et à utiliser dans les quatre jours." Dans le cas d'une solution pour injection intraveineuse, on supprime l'addition de bactériostatique: on opère aseptiquement, et stérilise simplement à la température de l'ébullition pendant 15 minutes.

Le nombre des solutés injectables inscrits à la Pharmacopée britannique de 1948 est devenu beaucoup plus considérable[22]. Les procédés de stérilisation sont variés: stérilisation à l'autoclave à 115 ou 116°C. pendant 30 minutes pour tous les composés peu altérables par la chaleur: autoclave ou filtration pour les tartrates doubles, le glucose, la caféine, etc.; chauffage à 100°C. pendant 30 minutes pour l'oxychlorure de bismuth; chauffage à 98 ou 100°C. pendant 30 minutes en présence d'un bactéricide, ou filtration, pour les alcaloïdes; filtration seule pour les

solutés très altérables (aneurine, héparine, iodoxyl, mersalyl). La tyndallisation a été abandonnée. L'abandon de la tyndallisation, et les autres modifications apportées aux techniques de stérilisation de la Pharmacopée britannique de 1932 à 1948, ont été la conclusion d'un grand nombre de travaux effectués surtout en Angleterre et aussi en Hollande et aux Etats-Unis. Ces travaux ont eu pour but: 1. de préciser la valeur du chauffage à 100°C. en vapeur fluente. La méthode a été reconnue impuissante à détruire les spores de divers anaerobies (B. botulinus, B. tetanus, B. sporogenes)[23,24]. 2. En ce qui concerne la tyndallisation, la plupart des bactériologistes modernes admettent l'interprétation de Tyndall et pensent que la méthode ne peut être efficace que si dans l'intervalle des chauffages les spores peuvent redonner des formes végétatives. Il en résulte que si ces spores sont dans un milieu non favorable à la croissance bactérienne—comme c'est souvent le cas des solutions pour injection—la spore risque de rester non transformée d'un chauffage à l'autre, et la méthode devient alors ineffective d'après de nombreux expérimentateurs (Hillen[25], Coulthard[26,27], Davis[28,29]). Il semble, en particulier, que la méthode soit impuissante à stériliser les préparations huileuses contaminées (O'Brien et Parish[30]).

3. La méthode qui, au contraire, s'est révélée comme la meilleure est le chauffage avec addition d'un bactéricide. Une température de 98 à 100°C. pendant 30 minutes est alors suffisante[31,32]. Les produits qui ont été trouvés les plus efficaces sont le chlorocresol (0·25 pour cent) et le nitrate phénylmercurique(0·002 pour cent)[33]. Cette méthode est la plus employée depuis de nombreuses années en Angleterre. Par contre, les pharmaciens français paraissent avoir conservé la conviction de l'efficacité de la tyndallisation, bien qu'à vrai dire cette opinion repose seulement sur une expérience empirique.

Mais nous voyons, de plus, apparaître à la Pharmacopée britannique de 1948, deux types de préparations injectables particuliers:

1) Les solutions injectables de certaines substances biologiques préparées aseptiquement dans l'industrie sont inscrites à la Pharmacopée, sans qu'on précise leur mode de stérilisation. C'est le cas de la solution d'insuline, d'insuline-protamine-zinc, et de suramine.

2) Les solutions de substances qui ne peuvent pas être conservées à l'état dissous, même en solution stérile à basse température. Dans ce cas, on prépare, dans des conditions aussi proches que possible de l'aseptie, le composé cristallisé pur. On le répartit en flacons stériles, en atmosphère privée de bactéries (voir plus haut) et on scelle les flacons avec une capsule de caoutchouc à sertissage métallique, ce matériel ayant été stérilisé au préalable. Au moment de l'emploi, on introduit, avec une seringue, le volume convenable du solvant choisi stérile (en général, eau distillée ou solution isotonique de chlorure de sodium) et l'injection est faite aussitôt. Cette technique était utilisée dès avant la guerre, dans le cas des composés arsenicaux antisyphilitiques; elle s'est généralisée depuis (pénicilline, streptomycine, acétylcholine, etc. . .). Ce mode de conditionnement a l'avantage de permettre des prélèvements successifs à la seringue.

A la Pharmacopée britannique, figurent 10 solutés injectables préparés de cette manière. Le Codex britannique de 1949[34] prescrit d'ajouter, dans ce cas, un bactériostatique au produit cristallisé, de manière que si des prélèvements successifs de solutions doivent être pratiqués, on soit à l'abri d'un risque de contamination. On a le choix entre cinq bactériostatiques dont les plus puissants sont les dérivés phénylmercuriques, agissant à 0,001 pour cent.

On recommande d'utiliser, comme solvant, de l'eau fraîchement distillée, et stérilisée aussitôt après.

D'autre part, le même Codex britannique de 1949 donne des indications précises sur la technique à utiliser pour la stérilisation par filtration des solutés injectables. On utilise la porcelaine poreuse, le verre fritté ou l'asbeste. Mais quel que soit le filtre choisi, il est nécessaire d'effectuer d'abord un *essai d'efficacité de filtration* par la technique suivante: On dilue 4 ml. d'une culture de 48 heures de *chromobacterium prodigiosum* sur bouillon nutritif, dans 100 ml. de même bouillon nutritif neuf. On dispose le filtre à essayer dans un ensemble clos qui sera stérilisé en *entier*. On filtre la suspension bactérienne, sous pression au moins égale à 400 mm. de mercure. On recueille aseptiquement 50 ml. de filtrat, en vase stérile. On scelle, à l'abri des bactéries de l'air, et on porte à 37°C. pendant cinq jours. Aucune culture ne doit apparaître. Pour l'emploi, le filtre (répondant à cet essai) doit être fixé à une fiole à filtration, munie d'un filtre pour rentrée d'air, et d'un dispositif permettant la répartition en ampoules du liquide filtré, à l'intérieur de l'appareil stérilisé, de façon qu'aucune contamination ne soit possible *après* filtration.

Il semble que la tendance moderne soit de généraliser dans l'industrie l'emploi de la filtration pour tous les solutés limpides. Cette technique exige des installations très soignées et un contrôle très rigoureux. En effet, il est indispensable d'effectuer alors *toujours* le contrôle bactériologique de la stérilité du soluté.

L'édition 1947 de la Pharmacopée américaine[35] préconise, de préférence, la stérilisation à l'autoclave à 115·5°C. pendant 30 minutes, ou 121·5°C. pendant 20 minutes, ou 126·5°C. pendant 15 minutes. (Remarquons que ces chiffres sont en désaccord complet avec ceux que nous indiquions plus haut sur la relation température-durée.) Dans le cas où la substance ne peut pas supporter une température de 115°C. on admet l'utilisation du chauffage à 100°C. pendant 30 minutes au moins, mais avec addition d'un bactériostatique. Enfin, le chauffage discontinu est admis dans le cas des solutions de substances fragiles. On opère à 60° ou 80°C. en répétant l'opération quatre à sept fois. Mais la Pharmacopée américaine exige alors l'addition d'un bactériostatique à une concentration suffisante, pour empêcher la croissance de *tous* les micro-organismes dans la solution, et cette pharmacopée ajoute que "ce procédé n'est pas une méthode sûre de stérilisation," et que "la filtration doit être appliquée à sa place chaque fois qu'il est possible."

Il nous faut faire une mention à part de l'évolution des techniques de stérilisation des solutés huileux. Il semble bien qu'on ait eu tendance à

admettre primitivement que ces solutés étaient *plus faciles* à stériliser que les solutés aqueux, tandis qu'on est maintenant d'avis contraire. En effet, au Formulaire des Hôpitaux militaires français de 1909, on prescrit de stériliser les solutions huileuses injectables par chauffage discontinu à 58° ou 60°C. pendant une heure, six fois de suite. Au Supplément du Codex français de 1926[36], on stérilise à 105° pendant dix minutes, alors qu'au Codex français de 1937 on stérilise à l'autoclave à 115° pendant vingt minutes. Enfin, les Pharmacopées britanniques de 1932 à 1948 exigent chaque fois qu'elle est possible une stérilisation à 150° pendant une heure[37,38]. Cela s'accorde avec les conclusions d'un grand nombre de travaux sur la nécessité d'une stérilisation en présence d'*eau*, pour obtenir la destruction rapide, à température relativement peu élevée, des formes végétatives bactériennes. Si les solutions huileuses sont rigoureusement anhydres, il est très certain qu'un chauffage à 150° est en effet nécessaire.

A cette question se rattache une difficulté pratique très importante: On admet facilement qu'un grand nombre de médicaments existant dans les solutés injectables ont par eux-mêmes une action antibiotique ou bactériostatique non négligeable, ce qui rend plus rapide la stérilisation par chauffage. On est même, dans certains cas, dispensé, de ce fait, d'ajouter un bactériostatique à la solution. Mais on pense moins souvent au cas de substances qui, au contraire, *protègent* la bactérie vis-à-vis de la stérilisation. Ces substances paraissent être surtout de nature lipidique, bien que jusqu'ici on ait à leur sujet des indications empiriques plutôt que des conclusions certaines d'expériences de laboratoire. On a rarement à examiner ce problème dans le cas de solutés injectables limpides, mais plus souvent dans le cas de solutés troubles ou d'émulsions constituées par des extraits de tissus ou de liquides biologiques. J'ai eu, pour ma part, à examiner une préparation contenant un extrait aqueux de rate tenant en suspension des lipides. Le produit avait été préparé presque aseptiquement et on se proposait ensuite de le stériliser. L'expérience a montré qu'un chauffage discontinu à 80° était *quelquefois* suffisant et *quelquefois* insuffisant, ce qui tenait au nombre inégal de germes bactériens existant initialement dans les divers lots de fabrication. Mais une stérilisation de 20 minutes à 100° pratiquée sur le même produit donnait des résultats également inconstants, et même une stérilisation à l'autoclave de 120° pendant 10 minutes, des résultats encore inconstants. La numération des bactéries avant et après stérilisation montrait qu'il y avait bien cependant *diminution* du nombre de germes après chaque chauffage, mais que cette diminution était très lente, par suite d'une protection manifeste des germes. Dans des cas de ce genre, seule l'addition d'un bactériostatique peut donner une sécurité relative.

## III.

### Contrôle Bactériologique de la Stérilité des Solutés Injectables

Ce qui a été exposé plus haut, de l'importance de la notion de probabilité en matière de stérilisation, fait comprendre qu'en cas de stérilisation insuffisante, le contrôle d'*une* ampoule ne pourra jamais suffire à prouver

la stérilité de tout un lot d'ampoules. Il faut au moins que l'essai porte sur une dizaine d'ampoules du même lot. Encore, la sécurité obtenue ne sera-t-elle pas très grande. C'est là une difficulté grave et qui a beaucoup embarrassé les techniciens chargés d'une expertise. Il existe d'ailleurs plusieurs autres difficultés supplémentaires :

1) Il est nécessaire de compter *tous* les germes présents, et pour cela les milieux nutritifs utilisés doivent permettre la croissance de *n'importe quel* germe;

2) Comme il est prescrit dans beaucoup de cas d'ajouter un bactériostatique aux liquides injectables, il faut que le contrôle de stérilité soit effectué dans des conditions où l'action du bactériostatique est négligeable.

Pour répondre à ces exigences, les prescriptions de la Pharmacopée britannique de 1948 sont les suivantes :

a) Emploi de milieux assez nutritifs pour permettre la croissance d'un seul germe (ou d'un très petit nombre de germes);

b) Essai avec un milieu neutre favorable aux aerobies et avec un autre milieu favorable aux anaerobies (Déjà la pharmacopée de 1932 prescrivait d'utiliser ces deux types de milieux);

c) On peut aussi employer un milieu unique additionné d'une substance qui abaisse le potentiel d'oxydo-réduction à un niveau suffisant pour permettre la croissance des anaerobies;

d) Dilution du soluté dans le milieu à un taux d'au moins 1 p. 100 de manière à réduire la concentration en bactériostatique éventuel à moins de 1/10.000.

La Pharmacopée américaine de 1947 perscrit l'emploi d'un liquide de culture dont il donne la formule :

| | |
|---|---|
| l-cystine | 0,05 |
| ClNa | 2,5 |
| Glucose | 1,1 |
| Extrait aqueux de levure de bière | 5. |
| Peptone pancréatique de caséine | 15. |
| Thioglycollate de sodium | 0,5 |
| Eau distillée | 1.000. |

Les quantités de prises d'essai à ensemencer sont précisées, ainsi que la durée de séjour à l'étuve.

En ce qui concerne l'interprétation, la Pharmacopée anglaise depuis 1932 demande qu'on examine les tubes après 5 jours de séjour à l'etuve à 37°C. Si aucun tube n'a cultivé, l'essai est négatif. Si on a obtenu *une* culture, on recommence l'essai avec le même nombre de tubes. Si on obtient encore *une* culture, on recommence une troisième fois. Si les trois essais ont donné chacun au moins une culture, ou encore si le *même* germe a été retrouvé dans *plus d'un* essai, le lot est rejeté. On voit donc qu'il est admis que la présence d'une culture isolée est insuffisante pour porter un jugement sur l'ensemble. La Pharmacopée américaine demande que pour les lots importants d'ampoules, on fasse porter l'essai sur 3 p. 100 du nombre total avec un maximum de 10, et elle demande de rejeter *tout lot ayant donné des cultures* après 7 jours de séjour à 37°C. Dans le cas où le soluté injectable rend le milieu trouble, on doit, après 7

jours de culture, réensemencer sur un nouveau milieu et lire le résultat après 3 jours de séjour à l'étuve, au moins.

Le contrôle de liquides injectables stériles est plus difficile encore dans le cas d'un liquide biologique prélevé aseptiquement, mais non stérilisé. C'est le cas du plasma humain[38] dont le contrôle est effectué par les Américains d'une manière très rigoureuse. (Cet essai ne figure pas à la Pharmacopée américaine.) L'essai étant pratiqué comme il a été dit plus haut, si plusieurs tubes correspondant à un même lot donnent des cultures, et que le *même* germe *non pathogène* apparaisse dans *deux* de *ces tubes*, le lot entier est à rejeter. Mais si les germes sont différents et qu'un nouvel essai de contrôle portant sur le même nombre de tubes soit négatif, le lot est utilisable. Par contre, si un seul tube donne *une* culture d'un germe *pathogène*, le lot entier est à rejeter.

Cette distinction entre germes pathogène et non pathogène paraît très judicieuse, et il est probable que c'est ce dernier type de contrôle qui est le meilleur, mais il a l'inconvénient de ne pouvoir être pratiqué que par un bactériologiste très expérimenté, et dans un laboratoire spécialisé.

## Conclusions

1. On voit que, dans l'état actuel de nos connaissances scientifiques, il n'est pas possible d'affirmer qu'un procédé de stérilisation de solutés injectables donne une sécurité *absolue*, quel que soit l'état bactériologique initial du soluté.

2. La tendance moderne est de préférer au procédé par chauffage le procédé par filtration, à condition qu'il soit pratiqué avec des précautions très rigoureuses et qu'on ajoute aux solutés un bactériostatique.

3. Mais alors le contrôle bactériologique de la stérilité doit être effectué, et son interprétation pose des problèmes extrêmement délicats.

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# RESEARCH PAPERS

## A NOTE ON THE DETERMINATION OF ALKALOIDS BY EXCHANGE OF IONS

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Received March 3, 1950

D. van Os has given a survey of the various methods for the determination of alkaloids[1]. One of us (A. Jindra[2]) has described the first experiments in which the chromatographic method of ion-exchange in determining alkaloidal salts and some galenicals containing alkaloids was adopted. Further experiments on this subject have been carried out at this Institute in accordance with our view that the determination of the physiologically active base is to be considered the only precise method. The results achieved can be summarised as follows.

We aimed at working out suitable semimicro- and micro-methods for the determination of the alkaloidal salts and galenicals of the Czechoslovak Pharmacopœia I using anion-exchange resin. According to Kunin and Myers[3] the mechanism of reaction in the chromatographic column when applying resin Amberlite IR4B must be considered as anion exchange, having two phases. With most of the salts the reaction was quantitative and the results were entirely satisfactory where both the methods were adopted. As the weighed quantities of the samples were too small it became necessary to determine the content of the base electrometrically. The larger quantities of active constituent used in the semimicro-method enabled a more accurate reading of the 0·01N hydrochloric acid consumed to be made and therefore, the results on the whole were more accurate. On the other hand the equivalence point could also be determined correctly by the micro-method, as the rapid change in the concentration of hydrogen ions at the stage when the process was about to reach the equivalence point, was relatively greater and more expressive with regard to the small amounts of base than in the semimicro-method.

As the data in the literature on the application of the quinhydrone electrode in titrations of alkaloids differ, it was tested in experiments as indicative electrode in direct titrations of alkaloids, when the solutions are very dilute and the dissociation constants low. In general it can be said that this did not work well. The results were mostly lower and they were correct only for arecoline, papaverine and pilocarpine, i.e. for very weak bases. Stronger bases, as ephedrine and the tropeines, could not be titrated at all, which is to be expected since the quinhydrone electrode gives correct results only at *p*H8, in buffered solutions at *p*H9. The results thus obtained correspond on the whole to the opinion of Kolthoff[5] and the experiments of Wagêner and Gile[6]. The glass electrode is very suitable and the results obtained by its application were correct, but the antimony electrode proved to be the best.

With certain alkaloids, however, the results obtained were not satisfactory when the standard method was applied hence the conditions of the experiment had to be changed.

Apomorphine, which turns blue in dilute ethyl alcoholic solutions owing to the presence of oxidation products, had to be titrated in an atmosphere of nitrogen.

Physostigmine showed higher results. In solutions it is unstable and its decomposition products are most probably the cause.

Lower results were obtained with ephedrine hydrochloride even under changed conditions of experiment. Probably the acid component, owing to the greater basicity of ephedrine, is much more bound than in the case of other alkaloids and the Amberlite IR4B is unable to separate both these components quantitatively. This proved to be still more evident with cotarnine hydrochloride, which is a salt of a strong quaternary base. The separation was incomplete and the results achieved were very low (approx. 50 per cent. of the theoretical results). It is quite probable that a suitable resin of stronger basic character will be found, which will enable these salts to be determined.

Standard methods were worked out for the following alkaloidal salts: — Arecoline hydrobromide, atropine sulphate, cocaine hydrochloride, codeine phosphate, homatropine hydrobromide, morphine hydrochloride, papaverine hydrochloride, pilocarpine hydrochloride, quinine hydrochloride, quinine sulphate, scopolamine hydrobromide, strychnine nitrate.

Methods were worked out for the determination of the alkaloidal drugs and galenicals of the Czechoslovak Pharmacopœia I. The material was sorted into several groups according to the character of the alkaloids contained therein. In most cases it was necessary to isolate the alkaloid to some extent before the actual process. In some cases this isolation was considerably simplified, in other cases no simplification of the official methods was achieved. But even in such cases some advantage in speeding up the process is found as much smaller quantities of sample and solvent are used.

In the course of the process the cause of the anomaly in the determination of tincture of nux vomica, which could be traced also with other galenicals as mentioned in the previous article, was cleared up. Some galenicals in rapid direct experiments, without the prior removal of accompanying substances, showed slightly higher results, some even multiple results. This is caused (a) by the presence of amines in the drugs, which pass with the alkaloids into the solution, and are titrated simultaneously (b) by the splitting of salts of alkalis contained in the drugs. Although this splitting due to the resin is small still it is noticeable and is more especially of consequence when smaller quantities of samples are used. In such cases the alkalinity of the eluate becomes greater, the smaller the weight of the sample and is, therefore, relatively greater than with larger quantities of sample. The galenicals contain not only neutral salts but especially easily hydrolysable ammonium salts, the splitting of which is facilitated by hydrolysis.

With cinchona bark and its galenicals (fluid extract and dry extract)

direct chromatography gives rather high results. A method for separating the accompanying substances has been worked out.

With ipecacuanha root and its galenicals (tincture) when chromatographing directly it must not be forgotten that inactive psychotrine is determined together with emetine and cephaëline; e.g. with the tincture the results were insignificantly higher, with the root the splitting of salts is evident from the unsatisfactory results (approx. 50 per cent. of the theoretical results). The method worked out will secure correct results.

For nux vomica seed and its galenicals (tincture and extract) a method for the determination of the alkaloids has been worked out.

For belladonna leaf and its galenicals (tincture and extract) the method worked out proves to be more advantageous than the officially adopted methods, chiefly because of its complete and rapid separation of chlorophyll and other accompanying substances.

## Summary

Following on work previously published, methods depending on ion exchange by chromatography with Amberlite IR4B have been worked out for the determination of alkaloids in the alkaloidal salts, crude drugs and galenicals.

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# THE INFLUENCE OF IODIDE CONCENTRATION IN THE IODIMETRIC TITRATION OF PENICILLIN

By T. Canbäck, I. Ehrlen, K. Ilver, F. Reimers and S. Wester

*Communication from the Scandinavian Pharmacopœia Council*

Received March 10, 1950

In the analysis of penicillins according to the method described by J. F. Alicino[1], the penicillins are decomposed in alkaline solution to penicilloinates which are titrated iodimetrically. It appears from the works available on the iodimetric titration of penicillin that the iodine consumption is dependent on *p*H. Titrating at *p*H 6·24, A. M. Wild[2] found an iodine consumption of *c.* 8·2 equivalents, while when titrating in acid solution, as described by Alicino and others, the consumption was almost 9 equivalents. V. Pedersen[3] investigated the dependence of the iodine consumption on the acidity at *p*H values between 1 and 8 and found a dependency as depicted in Figure 1. B. Örtenblad[4] repeated these experiments and arrived at a completely different result. He did not find the marked increase in iodine consumption at *p*H values between *c.* 2 and 4·5. A series of investigations were carried out at the instigation of the Scandinavian Pharmacopœia Council, Penicillin Sub-Committee, both in the laboratories of the Danish and the Norwegian Pharmacopœia Commissions, and also in Sweden in the Control Laboratories of the Pharmaceutical Society. In the first mentioned, Pedersen's results were confirmed, while Örtenblad's results were confirmed in the two last mentioned laboratories.

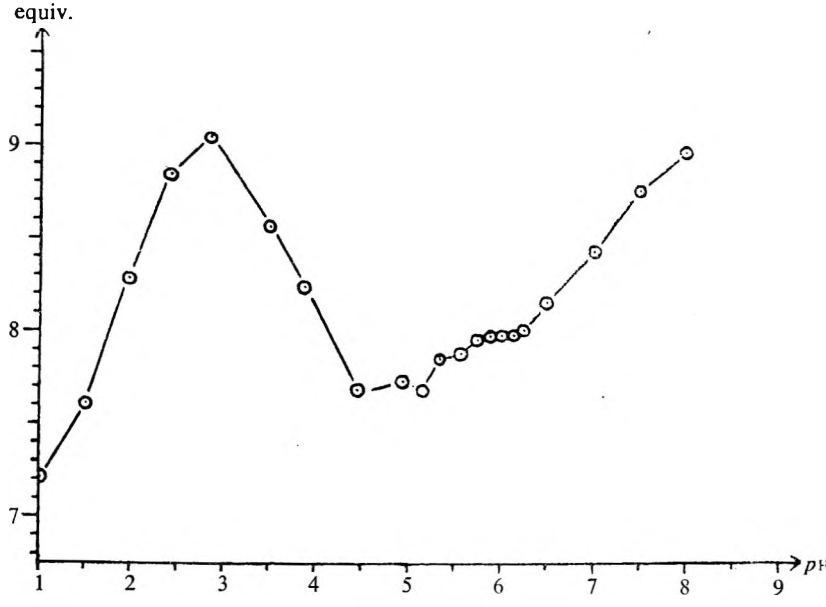


Fig. 1. Interdependence between iodine consumption and *p*H according to V. Pedersen[3].

In these experiments, the same analytical procedure and the same penicillin samples were used, and it is therefore obvious that the deviations must be caused by circumstances not prescribed in the outline of the analytical procedure. A detailed discussion of all experimental steps showed that the difference was probably due to the fact that the Norwegian and Swedish laboratories used 0·01N iodine containing 0·2 per cent. of potassium iodide, while in the Danish laboratory 0·01N iodine was used which was prepared according to Ph.Dan. 1948 and contained 2·2 per cent. of potassium iodide.

It has been found previously by Wild (*loc. cit.*) that during titration at *p*H 6·24 the iodine consumption decreases with increasing concentration of iodide. If the difference in iodide concentration should be the cause of the discrepancy between Pedersen's and Örtenblad's results, the effect of changes in the iodide concentration should be different at different *p*H values. The investigations into the influence of the iodide concentration carried out partly in the Control Laboratory of the Pharmaceutical Society in Stockholm, partly in the laboratory of the Pharmacopœia Commission in Copenhagen, have confirmed this assumption and also the fact that the disagreement found earlier must be a consequence of differences in the iodide concentration.

Our experiments were carried out in the following manner. The solutions used contained 0·0500 g. of benzylpenicillin salt in 100 ml.; 0·0800 g. in the case of procaine benzylpenicillin. The procaine salt was brought into solution either by shaking with water or by means of 1 ml. of methyl alcohol and subsequent dilution with water. To 5·00 ml. of penicillin solution 1·00 ml. of N sodium hydroxide was added and the penicillin decomposed by standing for 20 minutes. Then 5·0 ml. of buffer mixture, 1·00 ml. of N hydrochloric acid and 10·00 ml. of 0·01N iodine were added. After storage in the dark for 20 minutes at *c.* 20°C. titration was performed, using 0·01N thiosulphate with 2 drops of mucilage of starch as indicator. The blanks were carried through in the same way, however, omitting the treatment with N sodium hydroxide and the subsequent addition of N hydrochloric acid.

At *p*H values between 2·5 and 6, phthalate buffer (Ph.Svec. 1946) was used, and at *p*H values between 6·5 and 7 a phosphate buffer (Ph.Svec. 1946). In order to attain so high a buffer capacity that the *p*H would not change too much towards lower values during oxidation with iodine, we have used buffer mixtures of a concentration six times higher than stated in Ph.Svec. 1946. *p*H values 1·5 and 2 were obtained by addition of hydrochloric acid.

*p*H determinations on the titrated solutions were carried out by means of a glass electrode. The following penicillin samples were used:

(A) 2 samples of crystalline benzylpenicillin sodium purified from other penicillins, one by recrystallisation from butyl alcohol and acetone, the other by preparation through the di-*iso*propyl etherate.

(B) 2 samples of crystalline benzylpenicillin sodium—commercial.

(C) 1 sample of impure crystalline benzylpenicillin calcium.

(D) 2 samples of crystalline procaine benzylpenicillin—commercial.

The results obtained in the Danish laboratory and in experiments with

the Danish penicillins were in good agreement with the results obtained in experiments with Swedish penicillins, carried out in the Swedish laboratory. As examples of these results Figures 2 and 3 present curves

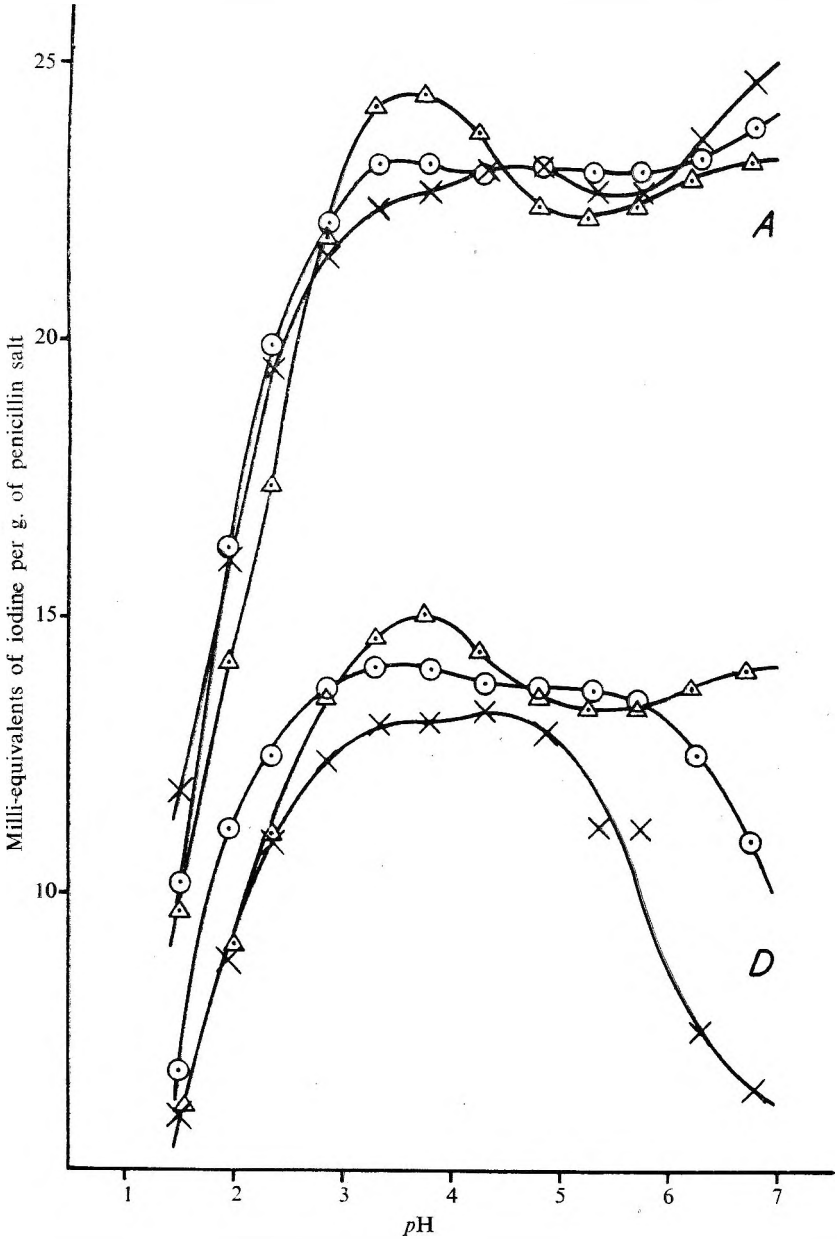


FIG. 2. Interdependence between iodine consumption and *p*H for pure crystalline benzylpenicillin sodium (A) and for crystalline procaine benzylpenicillin (D).
x——x 0·0125N KI in 0·01N I.
o——o 0·050N KI in 0·01N I.
Δ——Δ 0·200N KI in 0·01N I

showing the interdependence between iodine consumption (calculated as milli-equivalents of iodine per g. of penicillin salt) and *p*H for one preparation of each of the four groups A, B, C, and D determined with

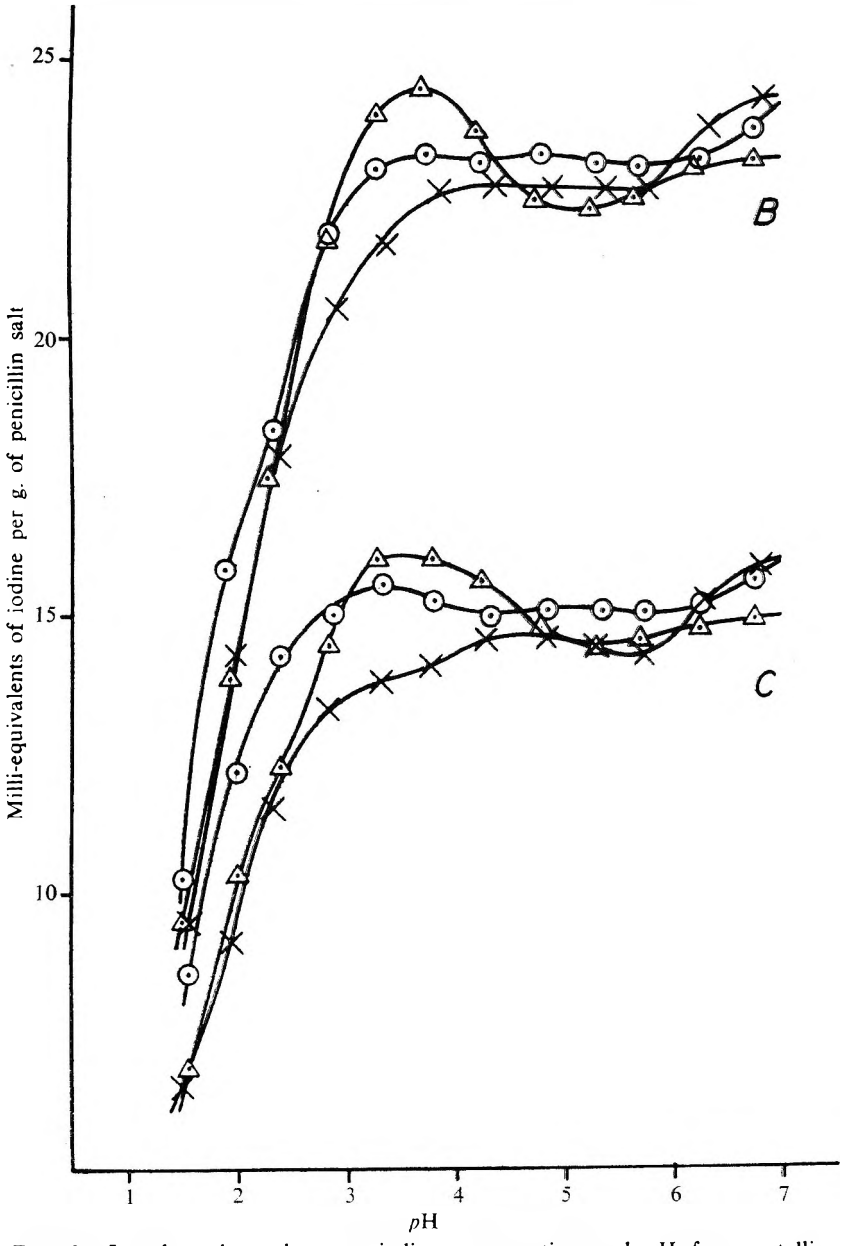


FIG. 3. Interdependence between iodine consumption and *p*H for crystalline benzylpenicillin sodium (commercial) (B) and impure crystalline benzylpenicillin calcium (C).

x——x 0·0125N KI in 0·01N I.
o——o 0·050N KI in 0·01N I.
Δ——Δ 0·200N KI in 0·01N I.

0·01N iodine, which was 0·0125N, 0·05N, and 0·20N, respectively, with respect to potassium iodide.

The curves of Figures 2 and 3 show almost the same effect of changes in the iodide concentration in experiments with the penicillin salts A, B, and C, while the course of the curve of the procaine salt D deviates at higher *p*H values.

In all cases, a significant increase in the iodine consumption as determined at a *p*H around 3·5 is found when 0·01N iodine with a high iodide

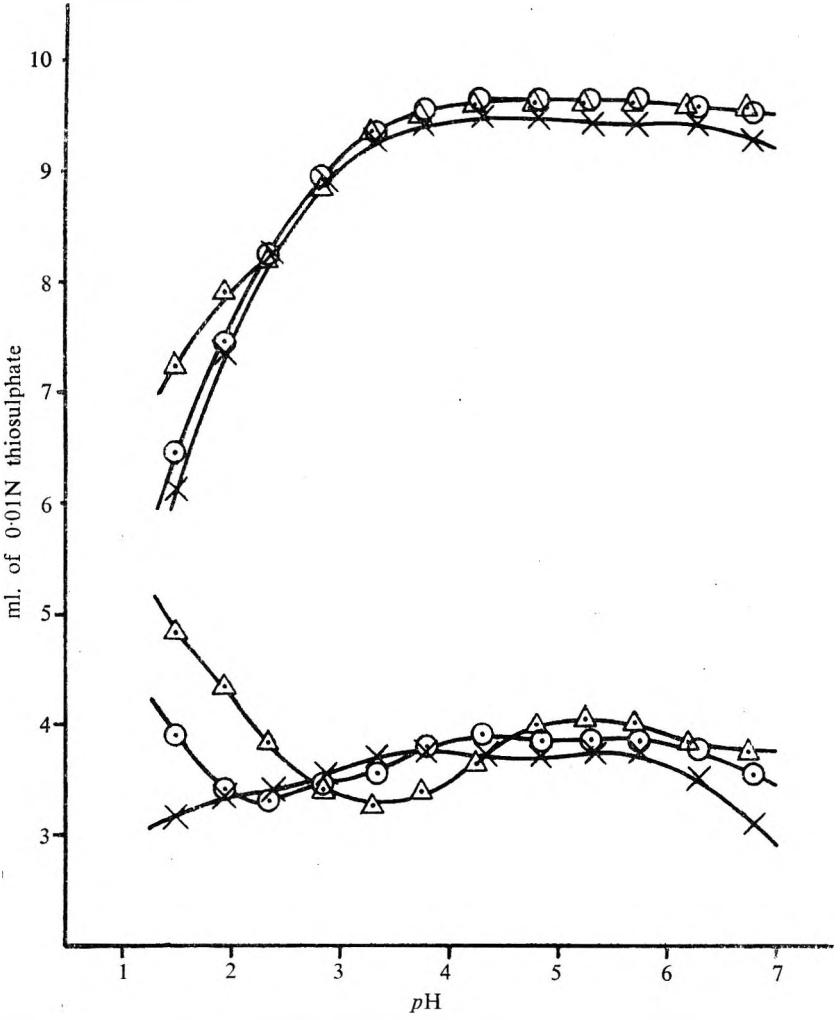


FIG. 4. Consumption of 0·01N thiosulphate in the analysis of 2·5 mg. of benzylpenicillin sodium (Upper graph).

Below: consumption by titration after treatment with sodium hydroxide.
Above: consumption when determining the blank.

x——x 0·0125N KI in 0·01N I.
o——o 0·050N KI in 0·01N I.
Δ——Δ 0·200N KI in 0·01N I.

concentration is used. At *p*H values below *c.* 3 the iodine consumption decreases, mainly because of the fact that the iodine consumption in the determination of the blank increases. This appears from Figure 4, which, as an example, shows the consumption of 0·01N thiosulphate in the analysis of purified benzyl penicillin sodium (A), both during titration of the penicilloic acids formed by decomposition and during the determination of the blank. Penicillin salts B and C lead to similar curves.

Analogously, the reduction in iodine consumption in the analysis of benzylpenicillin procaine at *p*H values above *c.*5, using 0·01N iodine with a low iodide concentration, must be due to an increased iodine consumption in the determination of the blank. Using 0·01N iodine which is 0·0125N with respect to potassium iodide, the titrated mixture turns opaque during determination of the blank. The colour change becomes somewhat less sharp when the determination is carried out at *p*H values above *c.*5. The same does not happen when the potassium iodide concentration is 0·2N and appears only at *p*H values above *c.*6 when the potassium iodide concentration is 0·05N. Opalescence does not appear when titrating the procaine salt after treatment with sodium hydroxide.

These experiments show clearly that it is necessary to prescribe the potassium iodide concentration in the titration solution to be used for the iodimetric titration of penicillin.

If it is desirable to apply a 0·01N iodine with a concentration of iodide in which a change of *p*H causes only a small change of the results, it is obviously not practical to use an iodide concentration greater than 0·05N while, on the other hand, for the titration of the procaine salt it is unfavourable to choose a concentration considerably below 0·05N.

It has been the purpose of the present investigation exclusively to show the interdependence between iodine consumption and *p*H when applying 0·01N iodine with varying iodide concentrations. It has not been the intention to determine the exact iodine consumption under different experimental conditions. For this reason we have not carried out control analyses of the penicillin samples applied in order to determine exactly their total content of penicillin.

## Summary

The potassium iodide concentration in 0·01N iodine used in the iodimetric titration of penicillin is of decisive importance for the result obtained. If it is desirable to use an iodide concentration for which small changes of *p*H involve only small changes of the result, it would not be practical to choose a concentration higher than 0·05N. At the same time, the iodide concentration used in the titration of procaine salts should not be considerably below 0·05N. These conclusions are based on the curves A—D of Figures 2 and 3.

We wish to express our thanks to Dr. B. Ortenblad for informing us of his results with the titrations of penicillin at different *p*H values, and

for his permission in the present paper to discuss his experiments which have not yet been published. We also wish to express our gratitude to Mr. P. Mørch and to Dr. H. F. Meldahl, who very kindly placed the samples of penicillin salts at our disposal.

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# EXPERIMENTS ON THE TOXICOLOGY OF 2 : 3 : 5 : 6-TETRACHLORONITROBENZENE

By G. A. H. Buttle and F. J. Dyer

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Received March 6, 1950

The experiments described were carried out to determine the toxic effects of 2:3:5:6-tetrachloronitrobenzene (T.C.N.B.) when given in varying levels in the diet of mice and rats. This material is being used for the treatment of potatoes to prevent sprouting and rotting, and it is important to determine whether any small quantity which may remain on the potatoes after they have been stored could be harmful.

## A. Chronic Toxicity in Mice

*Experiment No. 1.* Two groups of 12 mice each received a diet containing the substance over a period of 31 days. After a preliminary trial, dose-levels of 672 mg. and 67·2 mg./50 g. of diet were chosen and a third control group of 12 mice received 50 g. of unmedicated food daily. The food consumed by each group was determined by difference between the amount of food offered and the amount left over. Table I shows the total weight of food consumed by each group during the 31-day period, the average weight of food consumed daily per mouse and the average weight of drug consumed daily per mouse for each group.

TABLE I

Summary of food and drug consumption during 31-day mouse feeding tests

| Dosage mg. per 50 g. of Food | Total Food Consumed per Group (g.) in 31 Days | Average Daily Food Consumed per Mouse (g.) | Average Daily Drug Consumed per Mouse (mg.) |
|---|---|---|---|
| 672 mg. ... ... ... ... ... | 898 | 2·4 | 32·4 |
| 67·2 mg. ... ... ... ... ... | 1083 | 3·0 | 4·1 |
| Control—Nil ... ... ... ... ... | 1251 | 3·4 | — |

The average weights of the medicated and control mice were determined at the start of the experiment and at the end of the 31-day feeding period. Table II gives the values found and the calculated average gains in weight.

TABLE II

Average body-weight changes

| Dosage mg. per 50 g. of Food | Average Body Weight in Mice | | Average Gain in Weight (g.) |
|---|---|---|---|
| | At Start (g.) | At Finish (g.) | |
| 672 mg. ... ... ... ... ... | 18·1 | 18·1 | 0 |
| 67·2 mg. ... ... ... ... ... | 19·1 | 30·0 | 10·9 |
| Control—Nil ... ... ... ... ... | 21·6 | 32·3 | 10·7 |

The mice receiving the dosage of 672 mg. per 50 g. of food showed no gain in body-weight during the 31-day period; whereas the animals receiving 67·2 mg. appeared to gain weight at a rate approximately equal to that of the control group.

No unfavourable symptoms of toxicity were observed in either of the experimental groups. The animals were killed at the end of the 31-day feeding period and post-mortem examination revealed no detectable changes in the organs of either of the groups.

It, therefore, appears that 4·1 mg. of tetrachloronitrobenzene administered daily for 31 days to mice produces no detectable changes and has no adverse effect on the rate of growth as compared with that of the controls. A daily dose of 32·4 mg. definitely retards growth, but produces no other symptoms of toxicity and no detectable tissue damage.

*Experiment No. 2.* A group of 24 mice, weighing about 25 g. each received a diet containing 13·68 g. of the drug per 100 g. of food in an attempt to give each mouse 570 mg. per day. Due to reduced food intake in comparison with the controls, the treated mice took only 250 mg. of drug per day. A second group of 12 mice weighing approximately 19 g. each served as non-medicated controls. 5 of the 24 experimental mice died on the third and fourth days, and the experiment was therefore discontinued on the fourth day.

Post-mortem examination of the 5 dead animals showed fatty degeneration of the liver, and some fatty changes were also observed in the histological sections of the spleen and kidney.

The results of these two experiments are summarised in Table III. For simplicity the daily dose of drug has been converted to terms of a 20 g. mouse.

TABLE III

TOXICITY TO MICE

| Daily Dose mg./20 g. Mouse | Average Weight of Food Consumed per Mouse per Day (g.) | Number of Days on Diet | Results |
|---|---|---|---|
| 200 | 1·8 | 4 | 5/24 died of fatty degeneration of liver. |
| 35 | 2·4 | 31 | Growth completely inhibited. No other changes observed. |
| 4·3 | 3·0 | 31 | Normal growth. Average weight increase of 10·9 g. No other changes observed. |
| Controls | 3·4 | 31 | Average weight increase of 10·7 g. |

In each of the last three groups 12 mice were used and in the first group there were 24.

It appears from these results, that the daily consumption by mice of 4·3 mg. per 20 g. of body-weight (215 mg./kg.), has no harmful effects when given over a period of 31 days. A daily dose of 35 mg./20 g. of body-weight, or 1750 mg./kg., inhibited growth but caused no other

toxic effects. A daily dose of 200 mg./20 g. of body-weight or 10,000mg./kg., caused the death of 5 out of 24 mice in 4 days.

### B. Chronic Toxicity in Rats

The following experiment was carried out to determine the possible chronic effect in rats. For this purpose weanling albino rats received various amounts of drug in their diet for a period of 10 weeks. Observations were made on growth rate, and symptoms of toxicity such as weakness and diarrhœa were looked for. 30 animals were uniformly distributed in regard to litter mates, sex and initial body-weight into 3 groups of 10 rats each, with a further 10 for the control group. The animals were fed *ad lib.* and weighed every 2 days. The dosages for the medicated groups were 4 mg. in Group A, 20 mg. in Group B and 100 mg. in Group C per 5 g. of diet. Group D was the control.

The amount of food consumed by each group was determined, and the average dose consumed by each rat was calculated. The changes in body-weight over the 10-week period for the 4 groups and the corresponding amounts of food and drug consumed by the rats are summarised in Table IV.

TABLE IV

| | GROUP | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Average daily food consumption, g. ... ... ... | 8·5 | 8·5 | 2·0 | 8·5 |
| Drug administered, mg. :— | | | | |
| Per 5 g. diet ... ... ... ... ... ... | 4·0 | 20·0 | 100·0 | 0 |
| Mean daily intake ... ... ... ... ... | 6·8 | 34·0 | 40·0 | 0 |
| Per kg. body-weight per day* ... ... ... ... | 57 | 400 | 1111 | 0 |
| Mean body-weight of Rats, g. :— | | | | |
| At end of week No.— | | | | |
| 0 ... ... ... ... ... ... ... ... | 41 | 39 | 41 | 39 |
| 1 ... ... ... ... ... ... ... ... | 36 | 34 | 31 | 34 |
| 2 ... ... ... ... ... ... ... ... | 46 | 44 | 28 | 53 |
| 3 ... ... ... ... ... ... ... ... | 74 | 47 | (28) | 76 |
| 4 ... ... ... ... ... ... ... ... | 87 | 60 | (28) | 90 |
| 5 ... ... ... ... ... ... ... ... | 108 | 69 | † | 110 |
| 6 ... ... ... ... ... ... ... ... | 126 | 80 | † | 124 |
| 7 ... ... ... ... ... ... ... ... | 143 | 95 | † | 140 |
| 8 ... ... ... ... ... ... ... ... | 161 | 103 | † | 154 |
| 9 ... ... ... ... ... ... ... ... | 176 | 118 | † | 172 |
| 10 ... ... ... ... ... ... ... ... | 197 | 131 | † | 196 |
| Average change in weight ... ... ... ... ... | + 156 | +92 | − 13 | + 157 |

* Calculated from the mean of initial and final body-weights. † All dead.

No deaths occurred among the rats in the Groups receiving 57 mg. and 400 mg. of drug per kg. of body-weight per day. 9 of the 10 rats in Group C receiving 1111 mg. per kg. per day died by the end of 3 weeks and the remaining rat died in the fifth week.

It will be noted that the rats in Group A, taking 57 mg./kg. of body-weight per day, gained weight at the same rate as the control Group D. Group B, which consumed about 400 mg./kg. per day, gained at a slower rate than the control animals and their total gain in weight was

only 67 per cent. of that of the control group. The animals of Group C receiving 1111 mg./kg. quickly lost weight and all were dead by the fifth week.

No toxic symptoms were shown by the animals of Groups A and D. At the dosage of 400 mg./kg. per day the rats showed no changes other than a staining of the fur on the belly indicating excretion of a pigmented substance in the urine. Those in Group C receiving 1111 mg./kg. of body-weight showed very obvious toxic symptoms. At the end of one week the animals had stained fur, some had diarrhœa and all appeared weak and sickly. All died by the end of the fifth week.

In summary, these experiments indicate that the oral ingestion of 6·8 mg. of tetrachloronitrobenzene daily by young rats, or an approximate dose of 57 mg./kg./day for 10 weeks, produces no reduction of growth or toxic symptoms. A dose-level of 34 mg./day or 400 mg./kg./day caused a diminished rate of growth, but no other toxic symptoms and no deaths, but a dose-level of 1111 mg./kg./day produced a rapid loss of weight and the death of all animals within five weeks.

### C. Inhalation Tests

It was considered desirable to test the possible hazards arising from the inhalation of the powder, in order to assess the risks to which workers might be exposed while engaged in dusting potatoes and inhaling air heavily laden with the dust.

Six young rats weighing from 40 to 50 g. were divided into 2 groups so that litter mates were present in each group. All animals were subjected to a stream of air blown from an air pump for a period of half-an hour, 3 times daily for 10 days. The rats in the first group served as controls and were exposed to pure air only. The animals in the second group received air which was blown over finely powdered tetrachloronitrobenzene. The increase in weight of the treated rats was similar to that of the controls and the treatment with tetrachloronitrobenzene dust appeared to be without effect.

### D. Tests in Rabbits

Experiments were also carried out to test the possible effect of irritation of the eye and skin of rabbits.

No untoward reactions or symptoms of irritation were observed following instillation of the material into the eyes of rabbits each day for one week.

The pure compound was rubbed on the shaved skin of rabbits on 2 successive days. Blackening and induration of the skin were observed on the sixth day, but there were no general toxic effects. In further experiments with the material diluted in china clay to a concentration of 20 per cent. and 2 per cent. there was slight discolouration but no

induration. It appears that high concentrations of the substance caused some superficial change in the rabbits' skin, but no general effects.

## Conclusions

The experiments show that a daily dose of 215 mg./kg. of body-weight for mice and 57 mg./kg. for rats produces no ill-effect. On the other hand, dosages of 1750 mg./kg. of body-weight in mice, and 400 mg./kg. in rats cause an inhibition of growth rate.

Experiments on pigs Scorgie[1] indicate that the toxicity of the substance in these animals is of the same order as that in rats. So far as the work goes, therefore, there is no evidence of an increase in toxicity in the larger animals. How far the findings can be applied to effects of tetrachloronitrobenzene on man is at present a matter for speculation.

Acknowledgements are due to Messrs. Bayer Products, Ltd., for making available supplies of tetrachloronitrobenzene, which is incorporated at a strength of 3 per cent. in the commercial preparation, "Fusarex," used for the treatment of potatoes.

We should like to express our thanks to Mr. N. McLaren for his technical assistance.

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# THE COMPARATIVE ANTIBACTERIAL ACTIVITY OF *O*-CHLOROMERCURIPHENOL AND PHENYLMERCURIC ACETATE

By M. Woodbine*

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Received March 20, 1950

Of the organomercurial antiseptics, phenylmercuric acetate occupies an established place in both medicine and pharmacy and is favoured as a bacteriostatic agent of high efficiency and low relative toxicity. In common with other mercurial antiseptics, owing their mode of action to combination with sulphydryl groups, its functional efficiency may be revealed by discolouration of the product containing it by mercuric sulphide. Recently *o*-chloromercuriphenol appears to have established itself in spite of the lack of published work relating its worth to that of already established members.

The first reports of the antiseptic activity of *o*-chloromercuriphenol, synthesised by Whitmore and Hanson[1] and Neogi and Chatterji[2], were by Fargher, Galloway and Probert[3,4], who used it for protecting textile materials from fungoid attack. Hart and Anderson[5] showed that the mercury derivatives of hydrocarbons and phenols were effective bacteriostatic agents, claiming *o*-chloromercuriphenol to be the most powerful of a series studied and, later, reported on the bactericidal activity of its derivatives[6]. On the other hand, however, McClusky and Swingle[7] claimed that *sec*-amylcresol preparations were more effective than *o*-chloromercuriphenol, in the presence of plasma, against Gram-positive cocci. Its use in antiseptic paper and fabrics is the subject of a patent[8].

The antiseptic properties of the phenylmercuric salts, however, have been more extensively reported. Weed and Ecker[9], for example, reported on the utility of the nitrate, synthesised by Otto[10], owing to its lack of odour, colour, taste, staining and corroding properties, non-selectivity and non-inhibition in the presence of tissues and that aqueous solutions could be administered either *per os*, or parenterally. They also showed[11] that there was a decreasing activity from the nitrate through the chloride and bromide to the iodide. Birkhaug[12] confirmed Weed and Ecker's results on the nitrate, but Pyman and Stevenson[13] showed that the "nitrate" previously used was the basic salt "merphenyl nitrate," $PhHgOH.PhHg.NO_3$. Phenylmercuric acetate and nitrate were introduced as contraceptives by Baker, Ranson and Tynen[14]. Berry, Jensen and Siller[15] showed that 0·001 per cent. of phenylmercuric nitrate provides a wide margin of safety for sterilising thermolabile substances, a concentration safe for use, as shown by the toxicological studies of Wien[16]

* With the assistance of M. W. Cheeseman.

The practical work described in this paper was done at the Wellcome Research Laboratories, Beckenham, Kent.

while, according to Fust[17], 0·1 per cent. of phenylmercuric borate is adequate for the sterilisation of fæces, urine, sputum and linen.

## Experimental

Direct comparisons of the traditional type between the two compounds are illustrated in Tables I and II. These are derived by exposing constant inocula of the selected organisms to falling concentrations which vary by increments of 10 to 100 per cent. Evidence of bacteriostatis, at dilutions no longer lethal, is seen with both compounds in the case of Gram-positive but not with the Gram-negative organisms.

TABLE I

INHIBITING CONCENTRATION, IN MG./100 ML. OF NUTRIENT BROTH MEDIUM, OF PHENYLMERCURIC ACETATE AND *O*-CHLOROMERCURIPHENOL WHEN EXPOSED TO CONSTANT INOCULA OF THE ORGANISMS AND INCUBATED AT 37·5°C.

| | | Inhibiting dilution in mg./100 ml. | | | |
|---|---|---|---|---|---|
| | | Phenylmercuric acetate | | *o*-Chloromercuriphenol | |
| | | 18 hours | 48 hours | 18 hours | 48 hours |
| *Streptococcus pyogenes*... | CN 10 | <0·00012 | 0·0012 | 0·0040 | 0·031 |
| *Staphylococcus aureus* ... | CN 491 | 0·00025 | 0·00195 | 0·0010 | 0·015 |
| *Eberthella typhosa* ... | CN 512 | 0·062 | 0·062 | 0·125 | 0·125 |
| *Bacterium coli* ... ... | CN 348 | 0·125 | 0·125 | 0·250 | 0·250 |
| *Pseudomonas æruginosa* | CN 200 | >0·250 | >0·250 | >0·250 | >0·250 |

TABLE II

INHIBITING DILUTION IN NUTRIENT BROTH AT WHICH GROWTH OF *E. TYPHOSA* OCCURRED AT $2\frac{1}{2}$ MINUTES BUT NOT AT 5 MINUTES AND THE RATIOS OF BACTERICIDAL ACTIVITY OF *O*-CHLOROMERCURIPHENOL AND PHENYLMERCURIC ACETATE IN TERMS OF PHENOL

| | Phenol | *o*-Chloromercuriphenol | Phenylmercuric acetate |
|---|---|---|---|
| Inhibiting dilution... ... ... | 1/100 | 1/800 | 1/3200 |
| Ratio with respect to phenol ... | 1 | 8 | 32 |

Much more information, however, may be derived from estimates relating the time of exposure to per cent. mortality, or its probit. In this way time-response curves may be derived and compared with the isolated observations on an unknown curve (Tables I and II). A convenient expression of the estimate is the LT50, or the time required to kill 50 per cent. of the inoculum (Withell[18]) and, where the slopes of the lines (b) are not parallel, use may be made of the concentration exponent (n) of Phelps[19] which expresses, numerically, the power to which the concentration must be raised in order to give comparable rate of kill.

In cases where potent antiseptics are compared difficulties arise in estimating the low orders of time involved. An obvious solution is the

choice, in the case of the mercurial antiseptics, of a resistant organism such as *Bacterium coli* (Stark and Montgomery[20]), grown on a synthetic medium to minimise the reversal effects of proteins (Anderson and Hart[6], and Mirimanoff and Masset[21]). MacLeod's medium[22], with the addition of 0·2 per cent. of vitamin-free casein hydrolysate and 1·5 per cent. New Zealand agar gave readily counted colonies of *Bact. coli* after 48 hours' incubation at 37·5°C.

A second difficulty, caused by "carry-over" of bacteriostatic concentrations, is met by the inclusion of specific reversal agents such as glutathione (Fildes[23]) and, of the substances available for this purpose, which include thioglycollic acid (Graydon and Biggs[24], Heinemann[25]), cysteine hydrochloride (Smith, Czarnetsky and Mudd[26]), cysteine hydrochloride and 2:3-dimercaptopropanol (B.A.L., or British Anti-Lewisite[27-30]), glutathione and 2:3-dimercaptopropanol are the most satisfactory (Table III).

TABLE III

ANTIBACTERIAL CONCENTRATIONS OF PHENYLMERCURIC ACETATE AND *o*-CHLOROMERCURIPHENOL IN THE PRESENCE OF FALLING CONCENTRATIONS OF POTENTIAL REVERSING AGENTS USING *BACT. COLI* AS TEST ORGANISM

| Concentration of reversing agent → | Inhibiting concentration in mg./100 ml. | | | | | |
|---|---|---|---|---|---|---|
| | 1/1T | 1/10T | 1/100T | 1/1M | 1/10M | None |
| *Glutathione* :— | | | | | | |
| Phenylmercuric acetate ... | > 2·50 | 1·25 | 0·31 | 0·075 | 0·018 | 0·0045 |
| *o*-Chloromercuriphenol ... | 2·5 | 2·5 | 0·62 | 0·15 | 0·037 | 0·009 |
| *Thioglycollic Acid* :— | | | | | | |
| Phenylmercuric acetate ... | <0·001 | 0·075 | 0·018 | 0·018 | 0·018 | 0·0045 |
| *o*-Chloromercuriphenol ... | <0·001 | 0·31 | 0·037 | 0·037 | 0·037 | 0·0045 |
| *Cystine HCl* :— | | | | | | |
| Phenylmercuric acetate ... | 0·62 | 0·075 | 0·018 | 0·009 | 0·002 | 0·0045 |
| *o*-Chloromercuriphenol ... | 0·31 | 0·018 | 0·009 | 0·0045 | 0·002 | 0·002 |
| *Cysteine HCl* :— | | | | | | |
| Phenylmercuric acetate ... | 0·15 | 0·075 | 0·018 | 0·009 | 0·0045 | 0·002 |
| *o*-Chloromercuriphenol ... | 0·62 | 0·009 | 0·009 | 0·018 | 0·0045 | 0·002 |
| 2,3-*Dimercaptopropanol* (*B.A.L.*):- | | | | | | |
| Phenylmercuric acetate ... | <0·001 | 0·62 | 0·31 | 0·15 | 0·037 | 0·018 |
| *o*-Chloromercuriphenol ... | <0·001 | 2·50 | 2·5 | 0·62 | 0·31 | 0·037 |

Calculation of the LT50 and concentration exponents using different organisms, substrates and temperatures justifies the conclusions that *o*-chloromercuriphenol is more active than phenylmercuric acetate in protein-free medium, but that this relationship is reversed in the presence of proteins (Table IV).

*Acute Toxicity.* The acute toxicity of the two compounds was determined by the intravenous and subcutaneous administration to groups of 10 mice of fresh solutions in distilled water. The estimates of LD50 were derived by plotting the logarithm of the dose against probit of mortality and the errors of the estimates evaluated graphically according

to Gaddum[31]. The results (Table V) show that, although phenylmercuric acetate is more toxic than *o*-chloromercuriphenol, the toxicities are of the same order.

TABLE IV

CONCENTRATION EXPONENTS FOR PHENYLMERCURIC ACETATE AND *o*-CHLOROMERCURIPHENOL UNDER DIFFERENT EXPERIMENTAL CONDITIONS

| Compound | Concentration | LT50 (sec.) | Concentration exponent (n) | Experimental conditions |
|---|---|---|---|---|
| Phenylmercuric acetate... | 1/100T<br>1/1M | 32·0<br>37·0 | 0·0631 | Using *Bact. coli*, a temperature of 16°C, sterile distilled water + 0·01 per cent. thioglycollic acid for dilutions, and broth agar, with 0·01 per cent. thioglycollic acid for the counts. |
| *o*-Chloromercuriphenol... | 1/100T<br>1/1M | 31·0<br>33·0 | 0·0271 | |
| Phenylmercuric acetate... | 1/10T<br>1/20T | 39·0<br>102·0 | 0·4175 | Using *Staph. aureus*, a temperature of 16·5°C., sterile distilled water for the dilutions and broth agar for the counts. |
| *o*-Chloromercuriphenol... | 1/10T<br>1/20T | 33·0<br>64·8 | 0·2931 | |
| Phenylmercuric acetate... | 1/100T<br>1/1M | 30·6<br>243·6 | 0·9001 | Using *E. typhosa*, a temperature of 16·5°C., sterile distilled water for the dilutions and broth agar for the counts. |
| *o*-Chloromercuriphenol... | 1/100T<br>1/1M | 31·2<br>99·6 | 0·5041 | |
| Phenylmercuric acetate... | 1/1M<br>1/10M | 225·0<br>264·0 | 0·0694 | Using *Bact. coli*, a temperature of 17·0°C., sterile distilled water for the dilutions and MacLeod's medium + agar for the counts. |
| *o*-Chloromercuriphenol... | 1/1M<br>1/10M | 33·0<br>123·0 | 0·5714 | |
| Phenylmercuric acetate... | 1/1M<br>1/10M | 172·0<br>180·0 | 0·0591 | Using *Bact. coli*, a temperature of 27·5°C., sterile distilled water + 0·01 per cent. glutathione for the dilutions and MacLeod's medium + agar + 0·01 per cent. glutathione for the counts. |
| *o*-Chloromercuriphenol... | 1/1M<br>1/10M | 212·0<br>512·0 | 0·2545 | |

TABLE V

TOXICITY OF PHENYLMERCURIC ACETATE AND *o*-CHLOROMERCURIPHENOL WHEN ADMINISTERED BY THE SUBCUTANEOUS AND INTRAVENOUS ROUTES

| Compound | Route | LD50 (g./kg.) | Limits of error (P=0·99) |
|---|---|---|---|
| Phenylmercuric acetate ... ... | intravenously<br>subcutaneously | 0·019<br>0·027 | 86—116<br>87—120 |
| *o*-Chloromercuriphenol ... ... | intravenously<br>subcutaneously | 0·023<br>0·036 | 84—117<br>93—116 |

## DISCUSSION

Solubility, odour, staining and corrosive action together with direct comparisons of antibacterial activity of the traditional type (Tables I and II) have been used as criteria for the evaluation of potential antiseptics. The differentiation of closely related antiseptics possessing similar properties and the comparative assessment of bactericidal action when they are rapidly effective in high dilution presents many problems, one of which, for example, concerns the extent to which the comparative bacteriostatic activity with constant inocula (Table I) is applicable to those ratios used in pharmaceutical preparations[32].

The greater amount of information which may be derived from estimates relating time of exposure to percentage mortality and its convenient expression in the LT50 is of particular use in evaluating functionally similar antiseptics. In those cases where the slopes of the regression lines are not parallel, the Phelps' concentration exponent provides information as to the comparable rate of increase in antibacterial action with increasing concentration. In the case examined here phenylmercuric acetate is superior to *o*-chloromercuriphenol, but the reverse occurs when the assessment is carried out in the absence of protein.

The problem of "bacteriostatic carry-over" may be met by the presence of specific reversing agents, contained in both the diluting and counting media. The insolubility of mercuric sulphide indicated that "thiol" compounds were potential inactivators in the case of the mercurial antiseptics and, of the several compounds examined, glutathione and 2:3-dimercaptopropanol are the most active. Glutathione is the more preferable of the two, however, owing to its greater convenience in manipulation.

Estimates of the acute toxicity (LD50) show that phenylmercuric acetate is more toxic than *o*-chloromercuriphenol by both the intravenous and subcutaneous routes. The toxicities are, however, of the same order and correlate with those of Wien[16] for the nitrate. The difference is unlikely to be of significance in pharmaceutical preparations incorporating either of the two compounds.

## Summary

1. Phenylmercuric acetate possesses a greater *in vitro* antibacterial activity than *o*-chloromercuriphenol.

2. Glutathione and 2:3-dimercaptopropanol (BAL) are more effective inactivators of mercurial antiseptics than thioglycollic acid and cystine or cysteine hydrochlorides.

3. The rate of increase of antibacterial efficiency with increasing concentration is high for both compounds, but phenylmercuric acetate is to be preferred in view of its greater activity in the presence of proteins.

4. The use of concentration exponents is suggested for the comparative evaluation of potential antiseptics of similar properties or chemical structure.

5. Phenylmercuric acetate is more toxic than *o*-chloromercuriphenol following intravenous or subcutaneous administration.

The author thanks Dr. G. Brownlee for his help in the preparation of the paper.

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# THE EVALUATION OF THE BACTERICIDAL ACTIVITY OF ETHYLENE GLYCOL AND SOME OF ITS MONOALKYL ETHERS AGAINST *BACTERIUM COLI*

## PART XI—DISCUSSION AND GENERAL INFERENCES

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Received October 17, 1947

### INTRODUCTION

THIS paper constitutes the last of a series dealing with the evaluation of bactericidal activity. In Part I[1] a description was given of a standardised technique based on the capillary-pipette roll-tube combination for counting viable organisms. Part II[2] concerned the study of the disinfection action of ethylene glycol monohexyl ether; there it was shown that the fundamental character of the survivor-time curve was asymmetrically sigmoid and that it changed gradually to a form convex to the axes as the severity of the lethal conditions was increased. An asymmetrical sigmoid curve is difficult to describe mathematically; for statistical treatment rectilinearity is highly coveted. Methods of treatment of data which have achieved so much success in the biological assays of pharmacologically active substances were used in Part III[3] to transform the raw survivor-time disinfection data to more suitable functions. From the probit-log survivor time curves which were constructed, it appeared that there was a rectilinear relationship (indicating normal distribution of resistances) over a considerable portion. This seemingly satisfactory condition was analysed statistically in Part IV[4]; the conclusion reached was that the linear relationship (in the range of probits 4 to 6) was not strictly tenable. Nevertheless, when probit-log time regressions were assumed linear, parallelism could be demonstrated (without involving serious error) between the regressions given by different concentrations as well as the same concentration of a disinfectant thereby enabling a characteristic regression coefficient to be assigned to every disinfectant-organism reaction (Part V[5]). In Part VI[6], the values of the coefficients of ethylene glycol and its monoalkyl ethers from methyl to hexyl at 20°C. have been calculated, and in Part VII[7], the values at 30°C.

In Part VIII[8] the advantages of using intermediate mortality levels for the comparison of bactericidal activity were discussed. The LT50 was used and its logarithm computed mathematically from the probit-log time regression equations.

Parts IX[9] and X[10] were devoted to discussions concerning the significance of the dilution factor $n$ possessed by a disinfectant and also the temperature coefficients $Q_{10}$ and $\theta$; the methods of their calculation were illustrated.

This final paper attempts to assess the value of the findings reported in the previous communications in the light of the principles underlying the methods of biological assay. The previously obtained results are

also used to show the connection between bactericidal activity and chemical structure of the compounds investigated.

*The method of biological assay.* In his introduction to Burn's book on the Methods of Biological Assay, Dale[11] formulated certain general principles involved in biological assay processes. These were as follows:

1. Measurements made by biological means, like every other kind of measurement, must be essentially comparative. No assay could have any serious value unless it was made with reference to an accepted standard.

2. The standard chosen for the comparative test should be, or should owe its activity to, the active principle for which the preparation was being assayed and the active principle in queston should be that to which the therapeutic value was due.

3. If the test measured the important active principle, the biological reaction employed need have no relation to the therapeutic effect.

4. The method of assay should eliminate, or estimate and allow for, the inevitable variation in the response of the test object, both the differences in sensitivity between one individual and another and the variation within a single individual from time to time.

In order to facilitate comparison of substances it is necessary to procure regressions of a function of the effect and a function of the dose, which may be considered linear and parallel. A satisfactory linear dose-effect regression has been established in many instances between the probit (a function of the percentage mortality) and the logarithm of the dose. The assay involves the use of the regressions from both substances and since it is commonly assumed that the behaviour of the test animal is the same towards the standard and the unknown, then any fluctuation in slope is considered due to animal variation if the slopes are not significantly different; a common slope, $b_c$, is then calculated by averaging the values of the two individual slopes. Each regression is then adjusted so that it passes through its respective $(\bar{x},\bar{y})$ point and so that the slope is equal to $b_c$. The horizontal distance between the two, now parallel, regressions is equal to $M$, the logarithm of the ratios of the potencies. Reading along the probit 5 line, $M = \log LD50_s - \log LD50_t$, where $s$ = standard and $t$ = test substance. Since the lines are parallel, the horizontal distance between them will be the same at all levels. The antilogarithm of $M \times 100$ = potency of the unknown as a percentage of the standard. Gaddum[12] gave full details of the mathematical treatment of the assays and showed how the limits of error may be calculated. Bliss[13,14,15,16] developed further the statistical analysis of the results and showed how the technique could be applied to other biological problems involving the estimation of the potency of toxic substances. Irwin[17] reviewed the statistical methods as applied to biological assays and gave an authoritative opinion on some of the more controversial points.

*The application of the principles of biological assay to the evaluation of bactericidal activity.* The evaluation of bactericidal activity falls into the category of biological assays. The Rideal-Walker test does not comply with the principles for such assays; for example, no attempt is made to ascertain whether the active principles of the standard and test substances may really be considered the same. Withell[18] has indicated

the manner in which the principles enumerated by Dale[11] may be applied to bactericidal assays. The choice of a suitable standard substance against which to assess the test material is extremely important. To raise the method above criticism the values of the slopes of the probit-log time regressions should be the same, indicating in most circumstances, that the active principles are identical and the modes of action, similar. In practice it is impossible to set up a standard substance for every disinfectant, but until appropriate investigations have been carried out with a number of germicides, it is difficult to visualise the size of the problem. The task is further enlarged by disinfectants behaving differently in various solvents.

*The limitations of the extent of the linearity of the probit-log time regression in disinfectant processes.* The survivor-time data of the disinfection reactions between ethylene glycol and its monoalkyl ethers against *Bact. coli* (which have been shown to be fundamentally sigmoid in character) have been plotted as probits against log time in the hope of procuring a linear relationship. Between the range of probits 4 to 6, statistical analysis has shown that linearity may be assumed without great error and this has enabled parallelism to be demonstrated within these limits, between the regressions from solutions of different concentrations of the same germicide. Nevertheless, the experimental evidence from this work and from the results of Jordan and Jacobs[19], points to a sigmoid relationship between probit and log time when the complete range of mortalities is considered; the position of the change of slope in the lower part of the curve is of some importance as on it depends the successful use of the time for 50 per cent. mortality (corresponding to probit 5) as the criterion for comparison of bactericidal solutions. In the results from the experiments of Jordan and Jacobs (*loc. cit.*) it was shown that this change in slope occurred at probit 4·6 thereby making it impossible to utilise the section between probits 4 and 6 for comparative purposes. When heat was used as the disinfectant, these authors found[20] that there was on rapid change in slope at the lower end of the curve, and that between probits 4 and 6, the regression was roughly linear. Further work must be carried out on other disinfectants with a variety of organisms to ascertain if there are any broad generalisations which can be made.

*The establishment and use of a standard slope.* When the common slope is used to adjust the regressions of both the standard and the test substances, the distance between the lines will indicate the relative potency of the unknown. Gaddum[12] discussed the errors associated with the different manners in which the experiment could be designed. If it is assumed that the average sensitivity of the organisms remains constant and also that the characteristic curve is fixed both in shape and position, then once the standard regression has been established (from many determinations) there should be no need to carry out a test on the standard preparation every time a comparison is made. An occasional check is all that is necessary. It is most unlikely that the experimental conditions can be reproduced exactly in every laboratory and hence the actual magnitude of the slopes will probably vary in each establishment. Nevertheless, since the assay will always be carried out under local

standardised conditions, the relative potencies obtained from different laboratories should be comparable. When no reliable standard regression is available, then a complete experiment with standard and test substance has to be performed; a common slope is then calculated on each occasion and the relative potencies computed.

In the evaluation of the relative potencies of germicides it would appear that the procedure of utilising an established standard regression offers many advantages for routine testing. The results in this thesis have shown that it is not difficult to reproduce the experimental conditions in the same laboratory; fluctuations in the slope are relatively small and not significant. A standard curve can therefore be established with some precision and tests with the standard substance simultaneously with the unknown are rendered unnecessary. Comparison with the potency of the standard substance will have to be carried out until sufficient number of observations on the standard have accumulated. The experiments are made more accurate by pooling the results from all the determinations as the number of tests increases.

Withell[16] employed these principles for the evaluation of bactericidal activity and found that when *Bact. coli* was the test organism, the relative potencies of 0·5 per cent. phenol and 0·05 per cent. *para*chlor*meta*cresol was 1·135. Withell believed that parallelism existed between the slopes of the two regressions but did not show statistically that there was no significant difference between the values of the slopes. When this work was commenced it was hoped that the slopes given by ethylene glycol and the members of the homologous series of its monoalkyl ethers would all be parallel, thereby enabling ethylene glycol to be used as the standard substance. It has been shown that this is not so and hence a legitimate comparison is not possible. No attempt has therefore been made to establish figures similar to those calculated by Withell, for the bactericidal solutions considered here.

*The concentration exponent and the temperature coefficient.* The determination of the relative potency of a substance in terms of a standard gives no information concerning the value of the concentration exponent and the temperature coefficient. These are additional factors possessed by a disinfectant and bear no relation to its inherent bactericidal properties. It is of considerable value to have knowledge of these constants as they can be used to calculate the relative disinfection action at any concentration and temperature.

*The use of different organisms in the evaluation of bactericidal activity.* It is an added advantage to test bactericidal activity against different representative organisms so that a wider knowledge of the efficiency of the disinfectant can be formulated. In counting techniques the choice of organisms is limited to those which do not clump in the presence of the disinfectant and from which it can be said that single discrete organisms give rise to single colonies. Withell[18] suggested that a sporing organism should also be used; Hobbs and Wilson[21] in their investigations on the disinfectant activity of caustic soda solutions included tests against the spores of *B. subtilis*. The choice of test organisms would be guided to a certain extent by the uses to which the

disinfectant would be put: for example, a wound disinfectant would have to be active against organisms not necessarily met by a germicide used for disinfecting drains and sewers.

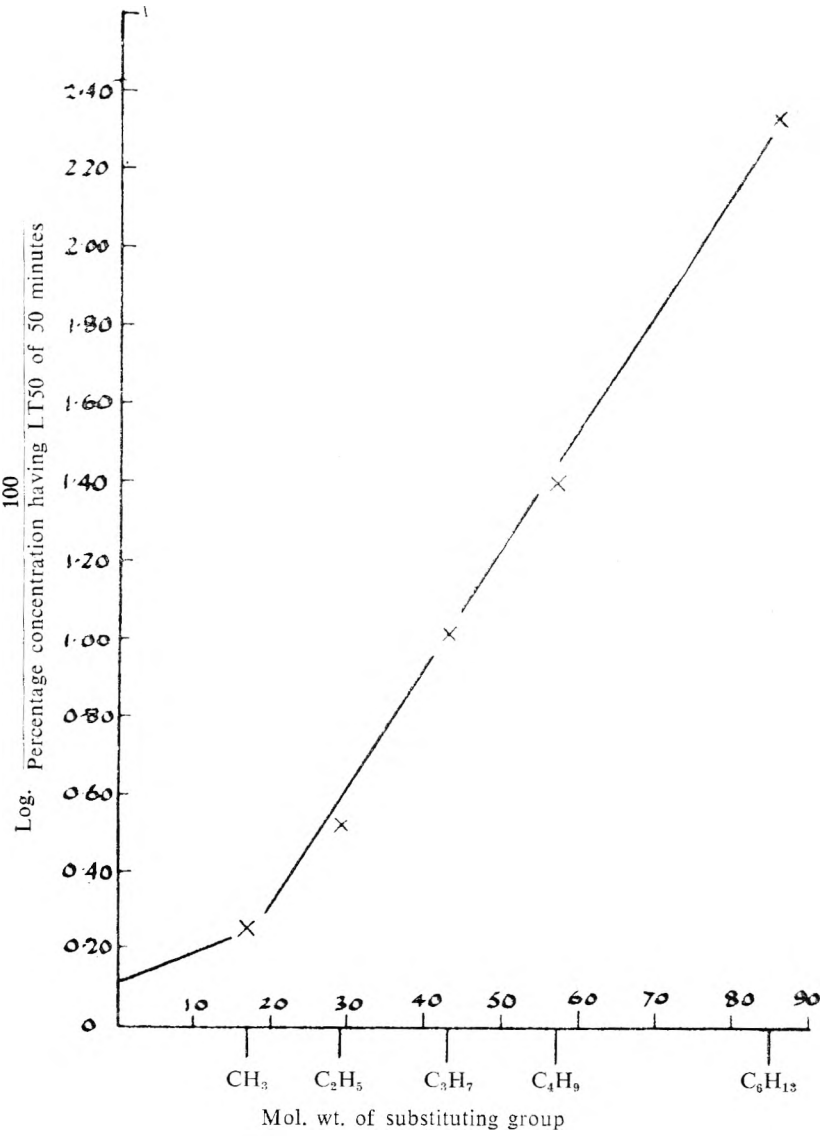


FIG. 1. Comparison between the variation in magnitude of the slopes of the probit-log. time regressions and the values of $n$ for members in the homologous series of ethylene glycol monoalkyl ethers.

*Variation in the magnitude of the slopes of the probit-log concentration regressions and the values of n for members in the homologous series.* The results enable a comparison to be made between the magnitude of the slopes of the probit-log time regressions and the values of $n$ for the

corresponding compounds. The figures, for the values at the two temperatures of the experiments, are set out in Table I and a graphical comparison is presented in Figure 1. The values of the slope for the probit-log time regression is out of line for ethylene glycol at 20°C. and for the monohexyl ether at 20°C. and 30°C. Nevertheless, it is clearly seen that as the magnitude of the slope rises to its maximum in the monopropyl ether, so the value of *n* falls to a minimum in the same compound. No explanation is offered for this phenomenon.

TABLE I

SUMMARY AND COMPARISON OF THE SLOPES OF THE PROBIT-LOG TIME REGRESSIONS AND THE CONCENTRATION EXPONENTS OF ETHYLENE GLYCOL AND ITS MONOALKYL ETHERS AGAINST *Bact. coli* AT 20°C. AND 30°C.

| Compound | At 20°C. | | At 30°C. | |
|---|---|---|---|---|
| | *b* | *n* | *b* | *n* |
| Ethylene glycol ... ... | 1·2025 | 15·8654 | 1·2938 | 18·4582 |
| Monomethyl ether ... ... | 0·7648 | 13·1874 | 1·3116 | 10·2271 |
| Monoethyl ether ... ... | 0·9242 | 10·5334 | 1·3412 | 6·2893 |
| Monopropyl ether ... ... | 1·7979 | 6·5400 | 2·1093 | 2·4849 |
| Monobutyl ether ... ... | 1·4176 | 10·0362 | 1·6054 | 4·0611 |
| Monohexyl ether ... ... | 1·6408 | 9·8170 | 2·0947 | 9·0755 |

(The values of the regressions at 20°C. are to be found in Table IX, Part VI[6], and those at 30°C. in Table X, Part VII[7] the values of *n* are given in Table I, Part IX[8]).

*Relationship between chemical structure and bactericidal efficiency.* It is clear from the summary of the results in Tables 2 and 4, Part VIII[8], that bactericidal efficiency increases as the homologous series of the monoalkyl ethers is ascended. In the calculations, however, percentage

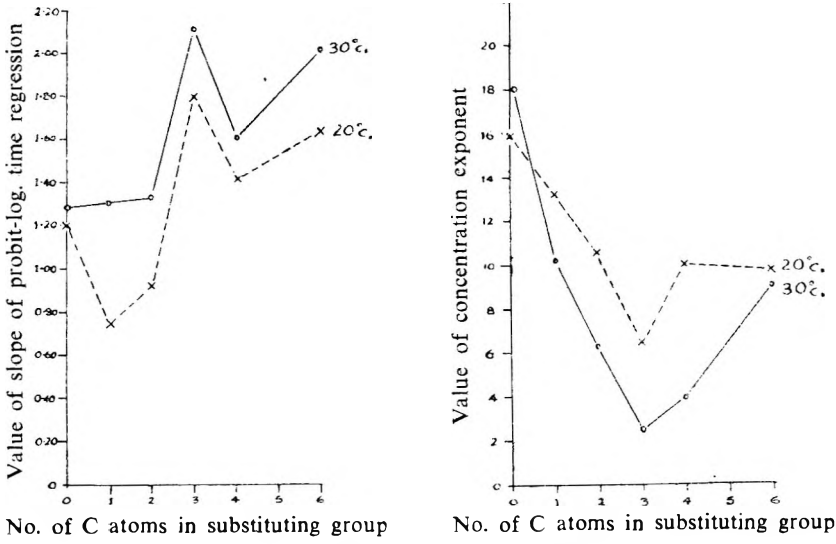


FIG. 2. The relation between chemical structure and germicidal efficiency of ethylene glycol and its monoalkyl ethers. Test organism: *Bact. coli.*

concentrations were used to present the strength of the disinfectant solutions; molecular concentrations are far more informative for comparative purposes. Bactericidal efficiency in aqueous solution is dependent on the substance being sufficiently soluble to be able to exert its activity. It is well known that members of such a series become less soluble as the number of carbon atoms in the chain is increased; it is unfortunate that the monoamyl ether is not sufficiently soluble for its solutions to possess a germicidal effect comparable with those of the remaining members. In order to compare the efficiencies of the members of the series, that concentration of each substance having an (arbitary) log LT50 of 1·699 (i.e., an LT50 of 50 minutes) was calculated from the respective log LT50-log percentage concentration regressions at 20°C. (Table Ia, Part IX[9]). The reciprocal of these concentrations was then multiplied by 100 to give a more manageable figure (Table II). The logarithms of these figures were then plotted against the molecular weights of the substituting alkyl groups (Fig. 2). It is seen that the points fall on a straight line with the exception of the ethylene glycol. Prolongation of the regression line backwards gives approximately $-0.225$ as the value of log (100 per cent. concentration) for ethylene glycol; for an LT50 of 50 minutes this would require a concentration of 167 per cent. Hence under the conditions of the calculations, ethylene glycol could not fall on the linear regression. Had a different temperature or different LT50 been chosen for the comparison, it is possible that an over-all linear regression might have been established.

TABLE II

RELATION BETWEEN CHEMICAL STRUCTURE AND GERMICIDAL EFFICIENCY OF ETHYLENE GLYCOL AND ITS MONOALKYL ETHERS

Test organism : *Bact. coli.* Temperature : 20°C.

| Compound | Log. concentration of compound having log. LT50 = 1·699 | Concentration per cent. | $\frac{100}{\text{per cent. concentration}}$ | Log. $\frac{100}{\text{per cent. concentration}}$ | Alkyl group | Mol. wt. of alkyl group |
|---|---|---|---|---|---|---|
| Ethylene glycol ... | 1·893 | 79·0 | 1·275 | 0·1055 | — | — |
| Monomethyl ether | 1·760 | 57·5 | 1·750 | 0·2480 | $CH_3$ | 15 |
| Monoethyl ether... | 1·474 | 30·0 | 3·330 | 0·5224 | $C_2H_5$ | 29 |
| Monopropyl ether | 0·956 | 9·0 | 11·100 | 1·0453 | $C_3H_7$ | 43 |
| Monobutyl ether... | 0·595 | 4·0 | 25·000 | 1·3979 | $C_4H_9$ | 57 |
| Monohexyl ether... | 1·665 | 0·46 | 218·000 | 2·3385 | $C_6H_{13}$ | 85 |

## SUMMARY

1. The principles underlying the methods of biological assay have been cited and reference has been made to their utilisation in the evaluation of bactericidal activity.

2. The importance of the choice of a standard disinfectant substance having a similar mode of action to the test substance has been emphasised.

3. The sigmoid nature of the probit-log time regression of disinfectant data (when the whole range of mortalities is considered) has been recalled and its bearing on the use of such a regression for the evaluation of bactericidal activity, has been discussed.

4. Although that part of the disinfection process between *Bact. coli* and its monoalkyl ethers from probits 4 to 6 of the probit-log time regressions has been taken as linear, it has been suggested that experiments be conducted with other organisms and disinfectants before broad generalisations be made.

5. It has been shown that constant sensitivity of the test organism (*Bact. coli*) towards the disinfectants could be reproduced readily; hence once the standard regression line had been confidently established, simultaneous tests with the standard and test substances were rendered unnecessary.

6. For a legitimate comparison to be made between the standard and test substances there must be parallel regressions. This desideratum could not be established in the experiments between ethylene glycol and its monoalkyl ethers; hence no comparison of the relative potencies of the compounds has been made.

7. The magnitude of the slope of the probit-log time regression rose to a maximum in the monopropyl ether, whilst the value of the concentration exponent fell to a minimum in the same compound, for experiments conducted at 20°C. and 30°C.

8. It was shown that bactericidal efficiency increased as the homologous series of the monoalkyl ethers was ascended. By plotting the logarithms of the reciprocal of 100 times the percentage concentration of each substance having an arbitrary log LT50 of 1·699 against the molecular weights of the substituting alkyl groups, a rectilinear relationship was obtained with the exception of ethylene glycol.

We would like to express our thanks to Dr. C. D. Darlington, Director of John Innes Horticultural Institution, for permission to use the calculating machines at his Institution. The statistical development of the work was directed by Dr. K. Mather, Head of the Genetics Department at the Institution; for his readiness to assist and for his clear guidance, we are deeply grateful. For technical assistance, we would like to thank Miss D. Alderton and Mr. J. W. Mackey.

This work forms part of a thesis submitted for the degree of Doctor of Philosophy in the University of London (I.M.).

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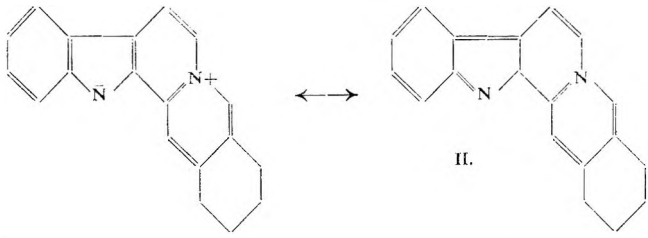
# ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

## CHEMISTRY

### ALKALOIDS

**Alkaloidal Titanifluorides.** M. Janot and M. Chaigneou. (*C. R. Acad. Sci., Paris,* 1949, **229,** 69.) The recent work showing that silicofluorides and zirconifluorides had formulæ different from those originally established by Schaeffer has been extended to include the preparation of titanifluorides. In the preparation of these salts, an aqueous solution of potassium titanihexafluoride $K_2TiF_6$ was mixed with an aqueous solution of the hydrochloride or sulphate of the alkaloid, when the titanifluoride crystallised out in the cold. Analyses of the salts showed the following formulæ: quinine, $B_2A,5H_2O$; quinidine, $B_2A,2H_2O$; cinchonine, $B_2A,H_2O$; cinchonidine, $B_2A,2H_2O$; strychnine, $B_2A,3H_2O$; brucine, $B_2A,6H_2O$; morphine, $B_2A.H_2O$; codeine, $B_2A$; narcotine, $B_2A$; thebaine, $B_2A,5H_2O$; corynanthine, $B_2A,4H_2O$; corynantheine $B_2A,H_2O$; where B is the alkaloid in question and A represents $H_2TiF_6$. The titanifluorides were all perfectly crystalline and could be used for microcrystalline identification of the alkaloids. R. E. S.

**Sempervirine, Structure of.** R. Bentley and T. S. Stevens. (*Nature,* 1949, **164,** 141.) The structure of sempervirine, the reddish-brown alkaloid of *Gelsemium sempervirens,* previously formulated by Prelog (*Helv. chim. Acta,* 1948, **31,** 588) as having two of its five condensed rings partially reduced, is now considered to be best formulated as an "anhydronium base" (I ⟷ II). Such a structure is considered to be consistent with the deep red colour and great strength of the base ($pK = 10{\cdot}6$).




the pale colour of the salts with acid, the formation of a methosulphate which on reduction with zinc and acetic acid gives a non-quaternary methyloctahydrosempervirine forming a benzyloiodide, and the formation of a benzylobromide which similarly reduced gives benzyloctahydrosempervirine forming the quaternary methiodide. Both nitrogen atoms are therefore involved in these changes. Dipole moment measurements were consistent with the expected high value for the proposed structure. F. H.

### ANALYTICAL

**Benzene Hexachloride, Spectrophotometric Determination of.** B. Davidow and G. Woodward. (*J. Assoc. off. agric. Chem.,* 1949, **32,** (4), 751.) The method described depends on the initial extraction of the insecticide from the material to be examined, the subsequent conversion of the benzene hexachloride with alkali to 1 : 2 : 4-trichlorobenzen , followed by the purification of this compound and its estimation by measurement of

the optical density at 286 mμ. Biological tissues are ground with anhydrous sodium sulphate to ensure dehydration and breaking up of the cells; the dried mixture (containing between 0·5 and 15 mg. of benzene hexachloride) is then extracted with ether. Dry materials, such as animal laboratory feeds, are ground and extracted directly without dehydration. The ether solution is evaporated and the residue refluxed with 20 ml. of a 1·5 N solution of potassium hydroxide in methyl alcohol for 1 hour; after cooling and adding 250 ml. of distilled water the mixture is extracted with 25 ml. of normal hexane and the hexane is washed with 10 quantities, each of 400 ml. of distilled water (without shaking). The hexane solution is dried by filtering through 10 to 12 g. of anhydrous sodium sulphate, which had previously been wetted with hexane, and the volume is adjusted to 25 ml. Most tissues needed no further purification but extracts from liver were purified by chromatography through a magnesium oxide column; extracts from laboratory diets were passed through a column of alumina. Hexane solutions of the unsaponifiable fractions of laboratory diets, spinach, potatoes, apple wax, and biological tissues, were found to have absorption properties in the ultraviolet region which interfered with a quantitative determination of benzene hydrochloride. To overcome this difficulty, absorption curves were prepared of the unsaponifiable fraction of these materials and on the products of alkaline hydrolysis of the isomers of benzene hexachloride. It was found that the spectral absorption curves of the control material were linear through the range of 284 to 290mμ. Resolution of the components contributing to the density at 286 mμ could be accomplished by two methods, the first consisting of a determination of the optical densities at 284 and 286 mμ and application of simultaneous equations for the resolution of a two component colour system. The second method used was the base line method in which the densities at 284, 286 and 290 mμ were determined. Recovery experiments showed a standard deviation of 8 per cent., the error being due to loss of benzene hexachloride and 1 : 2 : 4-trichlorobenzene because of their volatility, and to emulsification of a portion of the 1 : 2 : 4-trichlorobenzene. As little as 500 μg. of benzene hexachloride could be determined in biological tissues, in spray residues on vegetables such as spinach and cabbage, and in animal laboratory diets. R. E. S.

**Formaldehyde, Acidimetric Titration of, in the Presence of Ammonium Salts.** A. Casini. (*Ann. Chim. appl., Roma.*, 1949, **39**, 600.) Most pharmacopœias determine formaldehyde by oxidising it to formic acid. This method is unsatisfactory, as many side reactions, including the formation of hydrogen, make it inaccurate. The following method is based on the formation of hexamine with the quantitative liberation of acid according to the equation $6\ HCHO + 4\ NH_4Cl = (CH_2)_6H_4 + 6H_2O + 4HCl$. To 2 ml. of formaldehyde solution, add 2 or 3 drops of solution of methyl red (if necessary, neutralise exactly), 5 g. of ammonium chloride dissolved in 15 ml. of water and then 30 ml. of N sodium hydroxide. After thorough mixing, titrate the excess of sodium hydroxide with N hydrochloric acid until a permanent red colour is obtained; 1 ml. of N sodium hydroxide corresponds to 45 mg. of formaldehyde. H. D.

**Formaldehyde, Determination of.** L. Fiore. (*Ann. Chim. appl., Roma.*, 1949, **39**, 604). The method of the Italian Pharmacopoeia for the determination of formaldehyde depends on oxidation to formic acid by hydrogen peroxide and titration of the acid formed. This method is unsatisfactory, and 10 assays carried out on the same sample of formaldehyde solution gave figures varying from 28·35 per cent. to 34·95 per cent. The U.S.P. gives a similar method with modifications presumably aimed at producing

greater accuracy, but 10 assays on the same sample of formaldehyde used for the Italian method gave figures varying from 29·70 per cent. to 33·00 per cent., so the U.S.P. method is no better than the Italian and both are unsatisfactory. H. D.

**Phenazone, Alkalimetric Determination of.** F. Monforte and G. Stagno d'Alcontres. (*Ann. Chim. appl., Roma.*, 1949, **39,** 663.) When phenazone is heated with ammoniated mercury, a compound is formed which reacts with potassium iodide, combining with iodine and liberating alkali. This reaction can be used for the determination of phenazone. To a solution of 0·05 g. of phenazone in 400 ml. of water add 0·5 g. of ammoniated mercury and boil for about 15 minutes. Filter and wash the residue on the filter paper. Neutralise the filtrate, which still contains traces of ammonia, with 0·01N hydrochloric acid and, after cooling, add 8 g. of potassium iodide (quite neutral and free from iodate), then add 50 ml. of 0·01N hydrochloric acid and titrate the excess with 0·01N potassium hydroxide in the presence of phenolphthalein; 1 ml. of 0·01N hydrochloric acid is equivalent to 0·00188 g. of phenazone.

## ORGANIC CHEMISTRY

**Adrenaline, Fluorescent Oxidation Product of.** A. Lund. (*Acta pharmacol.*, 1949, **5,** 75.) The fluorescent substance is shown to possess the same oxidation step as adrenochrome; for its formation each molecule of adrenaline is oxidised by 2 atoms of oxygen. It is prepared in 65 per cent. yield by titrating an alkaline solution of adrenochrome with acid to *p*H 6·7. The crystalline precipitate thus obtained is separated by centrifuging, washed with water and dried. The fluorescent substance is yellow to orange-yellow, readily soluble in alcohol and pyridine, slightly soluble in acetone and water and insoluble in chloroform and ether. The aqueous solution is light yellowish green and strongly fluorescent. In crystalline form the fluorescent substance will keep for months, but in solution it is oxidised very rapidly. When heated to just above 100°C., the crystals become black without melting. The velocity constants for the formation of the fluorescent substance from adrenaline in alkaline solution and for the oxidative decomposition of the substance prepared as described were found to be the same and this was taken as good evidence for the identity of the two substances. G. R. K.

**Adrenaline-like Compounds, a Synthesis of.** G. Fodor and O. Kovács. (*J. Amer. chem. Soc.*, 1949, **71,** 1045.) A new synthesis of hydroxyaryl *N*-alkylamino ethanols is described. Hydroxyaryl methyl ketones and their esters or ethers were oxidised by means of selenium dioxide in good yields to the corresponding aryl glyoxals. On catalytic reduction in the presence of alcoholic methylamine or *iso*propylamine, the glyoxals were converted to the adrenaline-like aryl ethanolamines in good yield. The synthesis, *inter alia,* of 1-(2′-hydroxyyhenol)-2-methylamino-ethanol and of *N-iso*propyl-nor-adrenaline by this method is described. F. H.

**Phenazone, Iodomercuric Compounds of.** F. Monforte and G. Stagno d'Alcontres. (*Ann. Chim. appl., Roma,* 1949, **39,** 665.) When phenazone is boiled with ammoniated mercury and potassium iodide added various compounds are formed. The authors purified three, (i) $C_{11}H_{12}ON_2HgIOH$ which occurs in yellow scales, soluble in absolute alcohol, m.pt. 135°C.; it does not combine with further iodine. (ii) $C_{11}H_{11}ON_2HgI$ which occurs in white crystals m.pt. 160° to 161°C.

soluble in alcohol and acetone; it combines with iodine, (iii) $C_{11}H_{12}ON_2$-$HgI_2,H_2O$ which occurs in white needles m.pt. 159° to 162°C.; it combines with iodine. These substances are prepared as follows. Add 8 g. of ammoniated mercury in very fine powder to a solution of 3·76 g. of phenazone in 400 ml. of water, boil for about 15 minutes, filter and cool the filtrate. Then add drop by drop a 3 per cent. solution of potassium iodide until no further precipitate is produced. Set aside to deposit and filter off. The filtrate is opalescent but clarifies on standing and is then evaporated by gentle heat when a heavy yellow oil separates. This is dried at 80°C. in an oven and dissolved in alcohol, from which compound (i) can be crystallized. The original precipitate is extracted with acetone, from which compound (ii) can be crystallised. If the original precipitate is extracted with much cold alcohol and the liquid slowly evaporated in the cold, compound (iii) crystallises.

H. D.

## PLANT ANALYSIS

***Strychnos* species from British Guiana, Examination of.** H. King. (*J. chem. Soc.*, 1949, 955.) The finely powdered barks of *Strychnos Erichsonii*, *S. Melinoniana*, *S. Mitscherlichii*, *S. diaboli*, *S. toxifera*, *S. guianensis* and *S. hirsuta* were slowly percolated with tartaric acid solution (1 per cent.) until the extracts no longer gave a reaction with Tanret's reagent or until the extracts were very pale. The reaction with Tanret's reagent with a portion of the total percolate gave an indication of the alkaloidal content. The extracts were tested for curare activity, on a neutralised portion of the solution, using the righting reflex of the frog. All the species examined, except one near *S. guianensis*, contained alkaloids. Of four containing alkaloids with curare-action, *S. toxifera* yielded the strongest curare preparations. *S. diaboli* yielded an alkaloid, diaboline, which though itself of little pharmacological activity gave an O-methyl ether, the methiodide of which had a curare potency by the rabbit head-drop test one fifty-fourth of that of *dextro*-tubocurarine chloride.

F. H.

# BIOCHEMISTRY

## GENERAL BIOCHEMISTRY

**Carotene Solutions, Stabilisation of.** M. L. Cooley. (*J. Ass. off. agric. Chem.*, 1949, **32**, 706.) The stabilising effect of mixed tocopherols on carotene solutions has been examined. Identical sets of solutions were stored in daylight (not direct sunlight), at room temperature (20°C.), in the dark at room temperature, and in the refrigerator; the tocopherols used were natural and contained 220 mg. of mixed tocopherols per g. The solutions were stored for 12 weeks, and at regular intervals the colour of each was measured in a spectrophotometer at 440 mμ. At the end of the 12-week period the solutions were analysed chromatographically followed by an assay using the method of Devlin and Mattill (*J. biol. Chem.*, 1942, **146**, 123). It was found that by incorporating 10 to 50 times as much tocopherols as carotene a light petroleum solution of carotene did not deteriorate in 12 weeks; without tocopherols similar solutions were stable only for 2 to 3 days. The use of more than 5 mg. of tocopherols per 100 ml. of light petroleum may produce a measurable colour which would be erroneously recorded as carotene. The destruction of carotene in higher potency solutions is not as rapid as in dilute solutions, possibly due to an antioxidant effect by the

carotene itself. Recovery of carotene from a solution which had been passed through a magnesia adsorption column was not complete although degradation products were removed. R. E. S.

**β-Carotene, Stereoisomeric Analysis of.** E. M. Bickoff, M. E. Atkins, G. F. Bailey and F. S. Stitt. (*J. Assoc. off. agric. Chem.*, 1949, **32**, 766.) Work has been done on the development of a comparatively rapid, reliable, liquid chromatogram procedure for separating β-carotene extracts into three fractions, each containing primarily only one of the three isomers, all-*trans*, *neo*-B, and *neo*-U, which constitute 95 per cent. of the β-carotene equilibrium mixture. In order to obtain a quantitative chromatographic procedure a study of the adsorbent, developer, adsorbate, size of column and method of packing was made and a method is described for the stereoisomeric analysis of β-carotene extracts by liquid chromatogram procedure into *neo*-B, all-*trans*, and *neo*-U fractions, followed by colorimetric analysis of the fractions, the method being sufficiently simple to make its routine application practical. Calcium hydroxide to definite specification is used as the adsorbent and a sufficient quantity of carotene solution is added to the dry column to give about 40 μg. of carotene. The chromatogram is developed with a solution of 1·5 per cent. of *p*-cresyl methyl ether in light petroleum (b.pt. 88° to 90°C.) and maintained at an absolute pressure of 100 to 200 mm. Hg., the eluate being collected directly in 25-ml. volumetric flasks. The bands are eluted from the column in the following order: *neo*-β-carotene B, all-*trans*-β-carotene, *neo*-β-carotene U. The *neo*-B fraction contains all the eluate leaving the column before the leading boundary of the all-*trans* zone reaches the bottom of the column; further eluate is collected as the all-*trans* fraction until the leading boundary of the *neo*-U zone reaches the column outlet. The elution of the *neo*-U fraction is accelerated by the addition to the column of 15 ml. of 5 per cent. acetone in light petroleum when the collection of the all-*trans* fraction is nearly completed. Each fraction is made up to volume and the optical density determined on an aliquot portion, the concentration of the various isomers being calculated from factors determined on solutions of the pure crystalline *neo*-B, all-*trans*, and *neo*-U isomers. The method should be applicable to the stereoisomeric analysis of β-carotene extracts from any source. Results are given of the application of the method to the analysis of an iodine-isomerised solution of β-carotene. R. E. S.

**1:5-Vinyl-2-thiooxazolidone, an Antithyroid Compound.** E. B. Astwood, M. A. Greer and M. G. Ettlinger. (*J. biol. Chem.*, 1949, **181**, 121.) Several foods were found to inhibit the uptake of radioactive iodine in a manner similar to that of antithyroid compounds, the most effective of these being rutabaga (yellow turnip or Swedish turnip), which was subsequently utilised for isolation of the active principle. Extraction of rutabaga was carried out as described previously, being followed during the early stages of purification by a simplified rat assay with $I^{131}$. Examination of the absorption spectra of concentrates showed a strong maximum at 240 mμ, of intensity parallel to antithyroid activity, and since most known sulphur-containing goitrogens are thioamides the ultra-violet absorption spectrum was used to control the final purification of the active principle. Details of the purification by chromatography and by crystallisation are given, this resulting in the isolation of the antithyroid factor as colourless optically active crystals, m.pt. 50°C.,$[\alpha]_D^{31°C.}$ − 70·5° (2 per cent. in methyl alcohol). Analyses and a molecular weight determination furnished the molecular formula $C_5H_7ONS$, the compound being a weak monoacid, $pK_a$ 10·5, without

basic properties, and stable in hot alkali but not in acid. The ultra-violet absorption spectrum in aqueous solution exhibited a single intense maximum at 240 mμ, log ε 4·18, shifted by alkali to 232 mμ, log ε 4·08. The physical properties of the compound suggested the presence of the grouping -NH-CS-O- final evidence in this direction being afforded by infra-red spectra studies which indicated that the substance was either 4- or 5-vinyl-2-thio-oxazolidone. The behaviour of the rutabaga compound on boiling with 4 N hydrochloric acid (the optical activity disappeared and ammonia could not be detected) indicated that the antithyroid factor was actually *l*-5-vinyl-2-thiooxazolidone; this structure has since been confirmed by synthesis. The thiooxazolidone content of seeds of plants of the mustard family was studied spectrophotometrically; it was not detected in any plant except those of the genus *Brassica*, and within that genus it was absent from the seeds of mustard and cauliflower. It was isolated from the edible root of rutabaga and white turnip in amounts which varied from 0·12 to 1·0 g. per kg. but it could not be detected in the edible portions of other *Brassica* vegetables, including cabbage, Chinese cabbage, kale, cauliflower, broccoli, mustard-greens, or horseradish root. The compound appeared to be present in the plant as a water-soluble, ether-insoluble precursor, from which the thiooxazolidone was formed apparently by enzyme action; on boiling the root or seed in water the enzyme was inactivated. When tested in normal human subjects, by the use of radioactive iodine the compound was found to have an antithyroid activity equal to that of thiouracil. R. E. S.

**Vitamin $B_{12}$ and Related Growth Factors, Paper Chromatography of.** W. A. Winsten and E. Eigen. (*J. biol. Chem.*, 1949, **181**, 109.) A paper chromatographic procedure for separating the six alternative growth factors for *Lactobacillus leichmannii* 313 as previously reported is described, as well as the method of recognising the position of the several factors on a chromatogram by use of *L. leichmannii* 313 as a microbiological indicator. The extension of the method to the examination of other natural materials revealed the existence of at least one other substitute growth factor replacing vitamin $B_{12}$ in the nutrition of the test organism. In the method described, 10 to 30 μl. samples of the solutions under examination (*p*H 5·0) were spotted on Whatman No. 1 paper strips. The chromatograms were developed overnight at room temperature with wet *n*-butyl alcohol; the strips were allowed to dry in air for 1 hour at 30° to 35°C., being laid on agar plates seeded with *L. leichmannii* 313. After leaching for 5 minutes the strips were removed and the plate was incubated overnight at 37°C.; the resulting zones of bacterial growth were light and it was necessary to hold the plate at an angle with a source of light to one side and behind it, when the zones were always sharply defined, well-formed ellipses. Several commercial parenteral liver preparations for use in the treatment of pernicious anæmia were examined by this technique in addition to condensed fish solubles, a commercial stomach concentrate intended for use in pernicious anæmia, corn steep liquor, a commercial trypsin, Wilson's liver powder 1:20, and an enzyme digest of sperm desoxyribonucleic acid. The slowest moving zone at the top of the strip had a double character, suggesting two substances; analysis of crystalline vitamin $B_{12}$ preparations and vitamin $B_{12}$ concentrates indicates that the doublet zone of growth may be due to two forms of vitamin $B_{12}$. Examination of corn steep liquor showed no vitamin $B_{12}$ doublet but four other growth factors for *L. leichmannii* 313; in addition to at least two forms of vitamin $B_{12}$, five other substitute growth factors were found to be present in various natural materials. Evidence obtained from analysis of an enzyme digest of desoxyribonucleic acid

indicated that the more rapidly moving growth factors other than vitamin $B_{12}$ were of desoxyriboside character. In a quantitative analysis of vitamin $B_{12}$ and of substitute growth factors in a parenteral liver preparation it was found that after chromatography there was somewhat more apparent vitamin $B_{12}$ activity per ml. at the top of the chromatogram, when compared with the value obtained on the whole preparation without chromatography. The bottom section of the chromatogram, containing as it did the three fast moving alternative growth factors, contributed an additional amount of apparent vitamin $B_{12}$ activity, which was only about 25 per cent. of the activity at the top of the chromatogram; in a total tube assay of an unchromatographed sample, the value obtained (0·67 per ml.) was not the sum of the activities contributed by the top and bottom sets of factors measured separately. R. E. S.

## BIOCHEMICAL ANALYSIS

**Adrenaline in Blood, Fluorimetric Determination of.** L. Lund. (*Acta Pharmacol.*, 1949, **5**, 231.) Adrenaline is oxidised by manganese dioxide to adrenochrome and this is rearranged to adrenolutine on addition of strong alkali and sodium ascorbate; the latter prevents further oxidation of the adrenolutine. Irradiation with ultra-violet light shows a yellowish green fluorescence the intensity of which is measured in Coleman's electronic photofluorimeter, model 12B, using Schlott's glass filter BG 12 as primary filter and Corning's glass filter 3486 as secondary filter. Fresh blood, mixed with heparin, is centrifuged to remove corpuscles; 5 ml. of plasma with 5 ml. of 0·2 N sodium acetate is filtered rapidly through a 7 mm. diameter column of alumina (1 g.), previously washed with 20 to 30 ml. of water to remove fluorescent substances and then with 5 ml. of 0·2 N sodium acetate. The column is washed with 5 ml. of sodium acetate solution and eluted with a mixture of 10 ml. of 0·2 N acetic acid and 10 ml. of water. The eluate is made up to 20 ml. To each of two portions of 9 ml. in centrifuge tubes 0·1 g. of manganese dioxide (washed free of fluorescent substances with acetic acid and water) is added, the tubes are inverted for 1 minute and centrifuged for 30 seconds at 3,000 revolutions per minute; the liquid is filtered through filter paper (washed free of fluorescent substances and dried). Two portions (A and B) each of 8 ml. of the filtrate are taken. To A 0·84 ml. of a 20 per cent. solution of sodium hydroxide is added, and 5 minutes later, 0·16 ml. of 1 per cent. solution of ascorbic acid. To B 1 ml. of a mixture of 1 ml. of 20 per cent. solution of sodium hydroxide and 2·20 ml. of 1 per cent. solution of ascorbic acid, is added. Solutions A and B are exactly alike except that the adrenolutine fluorescence in A has been eliminated by oxidation so that A gives the individual blank value for B. For concentrations above 10 μg. per cent. of adrenaline in plasma the accuracy is ±5 per cent.; for concentrations between 1 and 10 μg. per cent. it is ±10 per cent. G. R. B.

**Aneurine, Microbiological Assay of.** E. E. Fitzgerald and E. B. Hughes. (*Analyst*, 1949, **74**, 340.) A modification of the method of Sarett and Cheldelin (*J. biol. Chem.*, 1944, **155**, 153) depending on the destruction of the aneurine in an extract of the sample by a modification of the sulphite treatment of Schultz, Atkin and Frey and the use of this sulphite-treated extract to supplement the standard tubes is described, all tubes used in an assay thus contain the same amount of the sample and a comparison can be made between untreated extract and sulphite-treated extract plus standard aneurine, inhibitory or stimulating effects due to substances other

than aneurine being in this way counteracted. The method is described in detail and prevents the unsatisfactory drift in the values obtained at different sample levels, due to the stimulatory or inhibitory effect on *Lactobacillus fermenti* 36 of substances other than aneurine in the sample extract, associated with the process of Sarett and Cheldelin. Using pure aneurine solutions, it was found that the sulphite treatment destroyed about 95 per cent. of the activity, but when the solutions were autoclaved at 15 lb. pressure instead of being steamed for 30 minutes, 99 per cent. of the activity was destroyed in 15 minutes; if this modification of the sulphite treatment was applied to sample extracts supplemented with aneurine, residual activity was absent. *L. fermenti* shows very little growth in the first 20 to 24 hours of incubation in the basal medium to which the hydrolysis products of the aneurine has been added (sulphite treated yeast extract was actually used). After this period, the organism grows rapidly, reaching full growth in a further 40 hours. The growth in the first 20 hours, although small, was difficult to account for until it was found to be due to residual aneurine in the alkali-treated peptone in the medium. When the peptone was prepared by treatment with sulphite, a very low "blank" was obtained in the first 20 hours of incubation. Under normal assay conditions, that is, in the presence of about 0·03 μg. of aneurine per tube, the lag phase is about 6 hours, the logarithmic phase is passed in a further 10 hours, and after this growth continues more slowly. Thus, with an incubation time of 18 hours, growth is independent of aneurine degradation products, whilst the effect of intact aneurine is approaching its maximum. This work indicated that by treating natural products such as peptone and yeast extract with sulphite it should be possible to devise a simpler basal medium with a shorter list of synthetic supplements; this is now being investigated. The method has been applied to a wide range of food materials. The results have been satisfactorily reproducible and have agreed reasonably well with parallel chemical assays. R. E. S.

**Aneurine in Yeast and Yeast Products, Assay of.** A. Jones and S. Morris. (*Analyst*, 1949, **74,** 333.) A study has been made of the plate method of assay (*Analyst*, 1948, **73,** 334) using *Lactobacillus fermentum*. The final method adopted used the basal medium of Sarett and Cheldelin (*J. biol. Chem.*, 1943, **150,** 1) with the addition of sodium chloride and 1·5 per cent. of agar; the organism used, *Lactobacillus fermentum* P.36, was maintained on the medium of Cheldelin, Bennett and Kornberg (*J. biol. Chem.*, 1946, **166,** 779) and sub-cultured every 3 weeks. For the inoculum *L. fermentum* was grown for 18 hours at 37°C. in 10 ml. of basal medium minus agar, with the addition of 10 μg. of aneurine per ml.; the medium was centrifuged, the supernatant liquor discarded and the organisms washed twice with saline solution, being finally suspended in 20 ml. of sterile saline solution. In the preparation of the plates 5 tubes of medium were used for the standard and for each test sample, the contents of each tube being melted, cooled to 48° to 50°C., and 1 ml. of inoculum added; after thorough mixing the contents were poured into sterile Petri plates, allowed to set, dried for 1½ hours at 37°C. and stored until used, 2 to 3 hours later; 5 holes were cut in each plate, each hole 10 mm. in diameter. The yeast was prepared for assay by taking 1 g. and adding, with 0·05 g. of takadiastase, to 15 ml. of 1 per cent. sodium acetate buffer at *p*H 4·5. The *p*H of the mixture was adjusted to 4·4 to 4·5 with N acetic acid or sodium hydroxide as necessary, 2 drops of benzene were added, and the whole was incubated at 37°C. for 16 to 18 hours. After steaming for 10 minutes, cooling, adjusting to *p*H 6·5, diluting to 25 ml. with water and filtering, the filtrate was diluted 1:2, 1:4,

1:8 and 1:16; 0·1 ml. quantities of each dilution were then measured into the holes in each of the 5 plates. The assay range of 0·25, 0·50, 1·00, 2·00, and 4·00 μg. of aneurine hydrochloride per ml. was used for the preparation of the standard curve; 0·1 ml. of each standard solution was measured into the holes of each of the five plates which were then incubated for 18 hours at 37°C. with the lids raised. The effect of doubling the aneurine concentration was to increase the zone diameters by 2·0 mm., the following equation expressing the relationship: $\log D = aZ - b$, where $a$ and $b$ are constants, D is the aneurine concentration in μg. per ml. and Z the zone diameter in mm. corresponding to a yeast concentration of M mg./ml. With the given test conditions, $a = 0{\cdot}1505$ and $b$ is dependent upon the intercept of the standard curve for any given day. From this, the aneurine concentration in the yeast in μg. per g. is given by 1000 D/M. The method gave valid and accurate results, though it was not particularly sensitive. R. E. S.

**Ascorbic Acid in Blood, Determination of, by the Dinitrophenylhydrazine Method.** W. Daubenmerki. (*Acta pharmacol. toxicol.*, 1949, **5**, 270.) The colorimetric, photo-electric macro method for the determination of ascorbic acid in serum and in whole blood by means of dinitrophenylhydrazine (described by Roe and Kuether, *J. biol. Chem.*, 1943, **147**, 399, and modified by Lowry, Lopez and Bessey, *ibid.*, 1945, **160**, 609) is reliable both quantitatively and qualitatively. By controlled oxidation in presence of suspended charcoal, ascorbic acid is converted to dehydro-ascorbic acid, which forms an osazone with dinitrophenylhydrazine; the intensity of the stable, deep-red colour produced on addition of strong sulphuric acid is measured photo-electrically. It is asserted that in the given circumstances blood contains no substances likely to upset the specificity of the method. In recovery experiments, 0·2 mg. per cent. of ascorbic acid added to serum or citrated blood was recovered with an accuracy of 99 to 100 per cent. with a maximum deviation of ± 4 per cent. Comparison with Farmer and Art's method of titration with dichlorindophenol showed this to be grossly inaccurate. Favourable agreement was found between the dinitrophenylhydrazine method and a photo-electric dichlorindophenol method. Ascorbic acid is unstable in clotted blood. G. R. B.

**Aureomycin, Assay of, in Body Fluids.** H. D. Brainerd, H. B. Bruyn, Jr., G. Meiklejohn and M. Scaparone. (*Proc. Soc. exp. biol., N.Y.*, 1949, **70**, 318.) Serum or other body fluid is stored at −40°C. prior to testing, and sterilised if necessary by Seitz filtration. Serial dilutions are prepared in a nutrient broth containing 20 per cent. of ascitic fluid, and to 0·2 ml. of each dilution, 0·5 ml. of a broth suspension of a strain of β-haemolytic streptococcus (JB) is added. The mixtures are incubated at 37°C. for 18 hours and the highest dilution which shows no growth visible to the naked eye is noted. Control tests using aureomycin solutions are run simultaneously, and generally indicate that the end-point of the test corresponds to a concentration of 0·031 μg. of aureomycin per ml. After oral administration of 1 g. of aureomycin, the maximum serum concentration, varying from 0·6 to 2·5 μg. per ml., is reached in 4 to 6 hours. When the dose is repeated every 4 to 6 hours, the serum level is gradually increased. Similar or greater serum concentrations are reached 5 minutes after intravenous injection of 50 mg. The concentration decreases rapidly for the first hour and then more slowly. 50 to 200 mg., injected intramuscularly, rarely produces measurable levels in the serum. G. B.

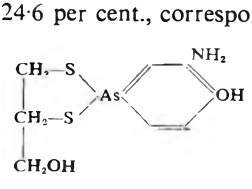
**Glucose, Galactose and Rhamnose in Mixtures, Determination of.** W. L. Porter and C. S. Fenske, Jr. (*J. Assoc. off. agric. Chem.*, 1949,

**32,** 714.) In studies of flavonol glycosides various methods, including filter paper chromatography, copper reduction and yeast fermentation were combined to determine the constituent sugars in hydrolysates of buckwheat and other plants. A filter paper chromatogram of the solution to be analysed was prepared and the identity of the sugars present was determined from the position of the spots. An aliquot of the original solution was diluted in the same manner as was done with the fermented samples and using Schoorl's method an aliquot of this solution was measured for total reducing sugars. If the chromatogram indicated that rhamnose was absent, but that either glucose or galactose or a mixture of these two was present, fermentation procedure A was followed; if rhamnose was present in combination with either or both of the two sugars, procedure B was used; if the chromatogram indicated only rhamnose, fermentation was unnecessary. Fermentation procedures A and B are given in detail and employed *Saccharomyces bayanus* (N.R.R.L. No. 966) for fermenting glucose and galactose. The fermentations when completed are followed by copper determinations of the undestroyed carbohydrate. From a combination of the results obtained at each stage the amounts of glucose, galactose and rhamnose can be calculated. Synthetic mixtures of glucose, galactose, and rhamnose could be analysed with recoveries of 98 to 104 per cent.; hydrolysates of a flavonol glycoside gave recoveries of approximately 96 per cent., which were probably low owing to destruction of sugar during the hydrolysis. R. E. S.

## CHEMOTHERAPY

**Amidone and Isoamidone, Homologues of, and some Related Compounds.** E. Walton, P. Ofner and R. H. Thorp. (*J. chem. Soc.*, 1949, 648.) In view of the established value of amidone, four series of related ketones were prepared by condensing diphenylmethyl cyanide with a chlorobase and treating the resulting basic cyanides with Grignard reagents. Corresponding amides, acids, esters and alkanes were also prepared. The compounds were examined for analgesic and respiratory depression activities. The two properties were always associated but not always in the same ratio, maximal activity being attained in the ethyl ketone in all the four series. 3-Dimethylamino-1 : 1-diphenyl-*n*-butyl cyanide was resolved by means of D-tartaric acid and from the optically active isomers the *dextro-* and *lævo-*amidones were prepared. F. H.

**Dimercaprol and Oxophenarsine Hydrochloride, Toxicity and Chemotherapeutic Effect of the Condensation Product.** J. L. Sawyers, B. Burrows and T. H. Maren. (*Proc. Soc. exp. Biol., N.Y.*, 1949, **70**, 194.) The equimolecular condensation product of oxophenarsine and dimercaprol is a light pink powder having m.pt. 122° to 124°C., and arsenic content 24·5 to 24·6 per cent., corresponding to a dithioarsenite of the following formula : —




It may be prepared by mixing a solution of 1·88 g. of oxophenarsine hydrochloride in 100 ml. of N hydrochloric acid with 1 g. of dimercaprol in 20 ml. of methyl alcohol, neutralising with sodium hydroxide, and washing the precipitate with water, methyl alcohol and ether. Compared with the original oxophenarsine hydrochloride, the therapeutic effect and toxicity for *T. equiperdum* in mice are both reduced, the former to a greater extent. The therapeutic index ($LD_{50}/CD_{50}$) for the dithioarsenite is 10, compared with 30 for oxophenarsine hydrochloride. A similar reduction in toxicity is observed in rats. It is suggested that the compound acts by dissociation to a

substance whose arsenic is available to the sulph-hydryl groups in protein, and that combination with dimercaprol alters the distribution of active arsenical between trypanosome and host. The hypothesis that the dithioarsenite acts as a whole cannot be explained by the -SH-arseno receptor theory. G. B.

***p*-Phenanthroline Derivatives as Antimalarials.** B. Douglas and W. O. Kermack. (*J. chem. Soc.*, 1949, 1017.) 4-Chloro- and 4:9-dichloro-*p*-phenanthroline were synthesised from ethyl ethoxymethylenemalonate and the chlorine atoms replaced by basic side chains. 9-Chloro-4-(3-diethylaminopropylamino)-*p*-phenanthroline and 4-(4′diethylamino-1′ methylbutylamino)-*p*-phenanthroline showed antimalarial activity of the same order as mepacrine and quinine against *Plasmodium gallinaceum* in chicks, delaying considerably the appearance of parasites in the blood in doses of 10 mg. per 20 g. body weight. F. H.

## PHARMACY

### DISPENSING

**Penicillin, Stabilisation of Aqueous Solutions of.** P. Fleury, G. Schuster, M. Dessus, J. Roux-Delimal and A. Morel. (*Ann. pharm. franc.*, 1949, **8**, 529.) Aqueous solutions containing not less than 20,000 Units per ml. may be preserved at room temperature for several months by the addition of 0·3 to 0·5 ml. of formaldehyde solution per 100 ml. On dilution with water, the potency of the solutions diminishes rapidly. Certain samples of solutions containing, in addition, citrate or phosphate buffers (*p*H 7·2 to 7·5) also deteriorate and the solutions may become slightly more acidic (*p*H not below 6·8). Solutions prepared with distilled water, glucose saline, or water treated with permutit, and containing phosphate buffer, formalin and penicillin (10,000 Units per ml.), all showed some deterioration when assayed microbiologically at intervals. The use of a solution of sodium hyposulphite, stabilised by borate, produced a stable preparation, possibly by preventing oxidation of the formaldehyde. The following method yields a solution stable at room temperature for several months. Dilute 0·2 ml. of neutralised formalin to 100 ml. with distilled water. Dissolve 100,000 Units of penicillin in 2·5 ml. of this solution, heat at 56°C. for 30 minutes and repeat the heating after an interval of 24 hours. Prepare a solution containing crystalline monopotassium phosphate, 0·25 per cent.; crystalline dipotassium phosphate, 1·56 per cent.; sodium hyphosphite (pentahydrate), 1 per cent.; sodium borate, 0·04 per cent.; sodium chloride, 1 per cent., and sterilise by autoclaving. Mix 2·5 ml. with the penicillin solution and store in ampoules. It has been observed that highly refined samples give less stable solutions, and further work is in progress. G. B.

**Penicillin G, Stabilisation of Aqueous Solutions of.** R. Paul, P. Gaillot and J. Baget. (*Ann. pharm. franc.*, 1949, **8**, 524.) The activity of aqueous solutions of penicillin G is rapidly destroyed by penicillinase and by heat. A sterile aqueous solution containing 200,000 Units in 20 ml. loses 90 per cent. of its activity on keeping at 25°C. for 4 days. The solution is most stable if buffered at *p*H 8·0 to 8·3. Citrate or phosphate buffers are compatible with penicillin G and well tolerated when given by injection, but citrates discolour on heating at 250°C. to destroy pyrogenic matter. The following is a suitable method for introducing the required buffer salts into containers and making a stabilised solution of penicillin G. Prepare a solution containing 0·1 g. mol. per litre of an equimolecular mixture of disodium

phosphate and monosodium phosphate, place 0·5 ml. in each container and heat at 250°C. for 1 hour to sterilise and to destroy pyrogen. Cool and add asceptically 200.000 Units of penicillin G dissolved in 20 ml. of sterile double-distilled water, isotonic sodium chloride or glucose solution. The deterioration of aqueous solutions of penicillin G is not accelerated by traces of alkali, and it is no advantage to use containers which comply with a limit test for alkalinity of glass. G. B.

## GALENICAL PHARMACY

**Ointment Bases, Absorption of Radioactive Sodium Iodide from.** G. N. Cyr, D. M. Skauen, J. E. Christian and C. O. Lee. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38,** 615, 618.) Ointments consisting of 10 per cent. of sodium iodide labelled with iodine$^{131}$ in lard, wool fat and soft paraffin were applied under rigorously standardised conditions to clipped areas of the backs of white rats. After 30 hours, in which time previous experiments had shown that maximum absorption took place, the animals were killed and their thyroids examined for content of radioactive iodine, using a Geiger Muller counter. Conversion of the results to percentages showed that the amount of applied iodine found in the thyroids was 0·045 per cent. from white soft paraffin, 0·041 per cent. from wool fat and 0·037 per cent. from lard. This technique was applied to 37 ointment bases. Whatever the base, there was some degree of absorption or penetration, the greatest amount (0·05 per cent. of iodide applied) being obtained with hydrous wool fat and the least (0·00006 per cent.) with a methylcellulose base. About 50 per cent. of the bases were more efficient than lard and the remainder less efficient. A difference in the type of emulsion, oil-in-water or water-in-oil, did not seem to exert any appreciable effect and with bases of the same type there was considerable variation in the amount of absorption. G. R. K.

**Sulphadiazine and Wetting Agents, Diffusion from Ointment Bases.** B. Levy and C. L. Huyck. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38,** 611.) The rates of diffusion of powdered and microcrystalline sulphadiazine from various ointment bases with and without wetting agents were compared *in vitro*. Results confirmed that water-soluble drugs should not be incorporated in water-in-oil emulsions since diffusion is greater from oil-in-water type ointments. Microcrystalline sulphadiazine was liberated faster and in greater amounts than ordinary powdered sulphadiazine from hydrophilic ointment U.S.P., and this rate was increased by the addition of the wetting agent, sodium lauryl sulphate. Microcrystalline sulphadiazine in white ointment U.S.P., with sodium lauryl sulphate, diffused slightly immediately after preparation, but did not diffuse after storage for 1 month at 37·5°C. Addition of sodium lauryl sulphate to white ointment containing 5 per cent. of microcrystalline sulphadiazine caused slight diffusion of the drug from the base. From hydrophilic ointment microcrystalline sulphadiazine diffused to a greater extent than ordinary sulphadiazine both at room temperature and at 37·5°C. G. R. K.

## PHARMACOGNOSY

**Anthraquinone Drugs, Colorimetric and Fluorimetric Studies on the Bornträger Reaction for.** B. V. Christensen and I. A. Abdel-latif. (*J. Amer. pharm. ass.*, 1949, **38,** 487.) The authors have re-investigated the published modifications of the Bornträger test and conclude that alcoholic potassium hydroxide is the best hydrolysing agent and that ether is the best

solvent for the liberated anthraquinones. The alcoholic potassium hydroxide extract of senna leaf when viewed under filtered ultra-violet light shows a strong pink fluorescence; during the test this fluorescence passes into the ether layer then into the ammonia layer with the anthraquinones. It is therefore suggested that the fluorescence is due to the anthraquinones and that it may be used as a sensitive test of their presence. The colour varies when other anthraquinone drugs are used in the test. Hydroxyl groups in the 1 and 8 positions of anthraquinone must be present in order that a positive Bornträger reaction as well as the fluorescence may be obtained. J. W. F.

**Cholla Gum.** F. Brown, E. L. Hirst and J. K. N. Jones. (*J. chem. Soc.*, 1949, 1761.) Cholla gum, an exudate of the cactus, *Opuntia fulgida*, has been shown to consist of L-arabinose (6 parts), D-xylose (2 parts), L-rhamnose (trace), D-galactose (3 parts) and D-galacturonic acid (1 part). Hydrolysis of the methylated derivative of cholla gum yielded 2:3:5-trimethyl L-arabinose (4 parts), 2:3:4-trimethyl D-xylose (2 parts), 2:3-dimethyl L-arabinose (1 part), 2:4-dimethyl D-galactose (3 parts), L-arabinose (1 part), and 2-methyl D-galacturonic acid (1 part). The L-arabinose residues were present in the furanose form. Cholla gum therefore resembled other plant gums in many features of its architecture, the main difference being that its acidity is due to D-galacturonic acid instead of D-glucuronic acid. F. H.

**Pyrethrum Flowers, Analysis of.** W. Mitchell and F. H. Tresadern. (*J. Soc. chem. Ind., Lond.*, 1949, **68**, 221.) It is shown that the assay of pyrethrum flowers using warm ligroin (Soxhlet) gives high results for pyrethrin II especially when the higher boiling grades are employed. The additional pyrethrins extracted with hot ligroin or chloroform were proved to be non-toxic to house-flies. It was also found that the additional matter obtained by hot extraction was insoluble in kerosene which is the basis of fly spray solutions. The use of cold ligroin (b.pt.: 40°to 60°C. or 20° to 40°C.) both for the assay and for commercial extraction is recommended. It is also suggested that the flowers should be standardised on their content of pyrethrin I only. G. R. A. S.

## PHARMACOLOGY AND THERAPEUTICS

**Antimalarial Drugs, Response of *Plasmodium berghei* to.** L. G. Goodwin. (*Nature*, 1949, **164**, 1133.) A new strain of *Plasmodium berghei* was used for the investigation of a series of 2 : 4 diaminopyrimidines, one of these compounds, 2 : 4-diamino-5-*p*-chlorophenoxy-6-methyl pyrimidine (48-210), being assayed simultaneously against *Plasmodium gallinaceum* in chicks and *P. berghei* in mice at the same dose-levels. Mice were inoculated intraperitoneally with a suspension of infected blood in heparinised saline solution, each mouse receiving *ca.* 5,000,000 parasitised erythrocytes; 6 doses of drug were given by stomach tube, night and morning for the following 3 days. Blood smears were prepared on the fifth and seventh days of the disease, and the percentage of parasitised cells determined for each mouse. Results are given in quinine equivalents and show that, although the quinine equivalents of 48-210 and chloroquine were about equal on the two species of *Plasmodium*, the relative activities of pamaquin and mepacrine were reversed: the low activity of pamaquin against *P. berghei* has been confirmed in further experiments, the variations possibly being due to differences in the rate of absorption and excretion of the drugs in mice and chicks, or by differences in the susceptibility of the parasites. R. E. S.

**Artane in the Treatment of Parkinson's Disease.** R. S. Schwab and W. R. Tillmann. (*New Engl. J. Med.*, 1949, **241**, 483.) Artane alone, and in combination with other drugs, has been used in 44 cases. Favourable results were obtained in 67 per cent. of cases in which artane was given. After 3 months' treatment only 7 of the patients were relieved sufficiently, as compared with their status on atropine, to remain on artane alone. The initial dose of artane is 1 mg. 4 times a day, and this dose is gradually increased until the patient is taking 3 mg. 5 times a day as a high level, the average being 2 mg. 5 times a day. Overdosage produces side-effects such as giddiness, dryness of the mouth, blurring of vision and headache. It was found less toxic than parpanit in older patients but less effective in reducing tremor; indeed, in some patients the tremor was worse in spite of reduction in rigidity. Nine of the patients required the addition of parpanit, and 7 were best regulated on a combination of atropine drugs and artane.

S. L. W.

**Atropine and Tripelennamine (Pyribenzamine) in Treatment of Peptone Shock.** J. C. Davis and H. O. Haterus. (*Proc. Soc. exp. Biol., N.Y.*, 1949, **70**, 275.) Intravenous injection of atropine (2 mg./kg.) of tripelennamine (10 mg./kg.) to anæsthetised dogs prevents death from shock subsequent to the rapid intravenous injection, 15 minutes later, of a 20 per cent. solution of Witte's peptone (5 ml./kg.). Neither substance prevents the initial fall in blood pressure, but there is a more rapid recovery of pressure in the animals protected by tripelennamine. A combination of atropine with tripelennamine appears to have no advantage over tripelennamine alone. In the concentration administered, atropine antagonises acetylcholine but not histamine, and so the results confirm that acetylcholine, as well as histamine, is concerned in peptone shock. G. B.

**Curare Assays; a modification of the Rat's Phrenic Nerve-Diaphragm Method.** G. B. West. (*Analyst*, 1949, **74**, 582.) Employing the usual method for the estimation of curare-like substances of suspending the isolated phrenic nerve-diaphragm in Ringer's solution at 37°C. the author was unable to obtain constant contractions for many hours, when using fluid electrodes and condenser charges from a neon lamp circuit. The temperature of the fluid was therefore reduced to 20°C. With a rate of stimulation of 8 stimuli per minute the tension developed varied from 1 to 10 g. and remained constant for several hours. When a dose of tubocurarine, sufficient to produce an inhibition of 50 per cent. of the muscle contraction in about 5 minutes, was added to the bath at 20°C. and the Tyrode solution changed after 5 minutes' action, the recovery of the preparation to its base-line required about 30 minutes. In an effort to reduce this time, potassium chloride was added to the bath of Tyrode solution. In small doses, up to 45 mg., this resulted in pure potentiation, while doses of more than 50 mg. resulted in initial stimulation followed by depression. It was found, however, that the tubocurarine must be removed from the bath before the potassium (40 to 45 mg. of potassium chloride) could aid recovery. This extra potassium was then washed out before the next dose of curare (100 µg. to a 75 ml. bath) was added. For the assay process, inhibitions of about 50 per cent. of the muscle contractions were produced, doses of the preparation under test being added at 8-minute intervals. With the lower temperature the diaphragm showed little sign of fatigue after 8 hours. A difference in activity of 7 to 10 per cent. could be detected by this means. S. L. W.

**Curarising Activity; Assay in the Conscious Mouse and Rat.** H. O. J. Collier, E. C. Fieller and R. A. Hall. (*Analyst*, 1949, **74**, 583.)

This is a report on methods used by the authors for the estimation of the activity of *d*-tubocurarine, and, in the rat, for the estimation of the dimethyl ether. The methods resemble in many respects those of Skinner and Young (*J. Pharmacol.*, 1947, **91,** 144), but differ essentially in the use of the intravenous route of administration. If a solution of *d*-tubocurarine of suitable concentration is given intravenously to a number of mice a proportion of them temporarily lose the righting reflex. Analysis of a number of experiments indicates that the log dose-probit line obtained by giving intravenous doses at several levels to groups of mice is both steep and straight, and this response may be made the basis of an assay method. The log dose-probit line obtained in a similar way with *d*-OO-dimethyl tubocurarine in rats is also steep and straight, and this response may also be made the basis of an assay method. S. L. W.

**Fat Emulsion, Intravenous Infusion into Human Subjects.** G. P. Shafiraff, J. H. Mulholland, E. Roth and H. C. Baron. (*Proc. Soc. exp. Biol., N.Y.*, 1949, **70,** 343.) An emulsion containing fatty oil (10 per cent.), protein hydrolysate (5 per cent.), glucose (5 per cent.) and gelatin (2 per cent.) and having a calorific value of 1300/1. and *p*H about 6·2 was prepared as follows. Refined coconut oil was autoclaved and mixed with sterile non-pyrogenic solutions of protein hydrolysate (amigen, 10 per cent.), glucose (50 per cent.) and intravenous gelatin (Knox P-20, 6 per cent.), and the mixture passed through a special homogeniser until the particle size of the emulsion was less than 1 μ. This emulsion was infused into surgical hospital patients in quantities of up to 16 l., at a rate of 20 to 80 drops per minute. The most severe reaction was a chill, observed in 9 per cent. of the patients and usually accompanied by a high temperature reaction. Allergenic reactions could be controlled by anti-histaminic drugs, and cough and vomiting were usually controlled by reducing the speed of administration. No pulmonary irritation, fatty emboli, serious toxic effects or late sequelae were observed. G. B.

**Methadone and its Isomers, Narcotic Power of.** J. E. Denton and H. K. Beecher. (*J. Amer. med. Ass.*, 1949, **141,** 1146.) The analgesic power of *dl*-methadone (6-dimethylamino-4 : 4-diphenylheptanone), *l*-methadone, *dl-iso*methadone (6-dimethyl-amino-5-methyl-4 : 4-diphenylhexanone-3), and *l*-isomethadone was compared with that of morphine in 429 patients with post-operative pain. The basis of comparison of the analgesic equivalence of morphine and the methadones was an arbitrary standard analgesic dose, the AD 90 per cent., which is defined as the analgesic dose giving moderate to complete relief of pain in 90 per cent. (limits 89 to 93 per cent.) of the trials. *dl*-Methadone and *l-iso*methadone are, mg. for mg., equivalent to morphine (AD 90 per cent. = 7 to 9 mg. /150 lb.) in analgesic power. *dl-iso*Methadone (AD 90 per cent. = 25 to 30 mg./150 lb.) is one-third as potent as morphine in analgesic power. *l*-Methadone (AD 90 per cent. = 4 to 6 mg./150 lb.) has approximately twice the analgesic potency of morphine and of *dl*-methadone. This suggests that the lævo-rotatory isomer has virtually all the analgesic power of the racemate and that the dextro-rotatory isomer is inactive. The reason that *l-iso*methadone has three times the analgesic power of its racemic form is not clear, but one possible explanation is that *d-iso*methadone has an anti-analgesic action. G. R. B.

**Quinidine Gluconate; an Intramuscular Preparation of Quinidine.** S. Bellet and J. Urbach. (*J. Lab. clin. Med.*, 1949, **34,** 1118.) Quinidine gluconate, $C_{20}H_{24}N_2O_2.C_5H_6(OH)_5COOH$, contains 62·3 per cent. of anhydrous quinidine and 37·7 per cent. of gluconic acid. It occurs as a white,

dextrorotatory powder, soluble 1 in 9 parts of water, giving a neutral or slightly alkaline solution. The solution is stable, and non-irritant. Observations on 15 patients showed that the quinidine effect appears in 15 minutes after a dose of 5 to 7½ gr. Absorption by the intramuscular route is certain, uniform and relatively safe, and its use is suggested for those patients in whom a rapid effect is required or in whom oral administration is not practicable. S. L. W.

**Thiomerin, Clinical Studies on.** I. W. Winik and R. B. Benedict. (*J. Lab. clin. Med.*, 1949, **34**, 1254.) Thiomerin, the di-sodium salt of N(γ-carboxymethyl-mercaptomercuri-β-methoxy)propyl camphoramic acid, is a new mercurial compound which has been shown by animal experiments to be much less toxic to the heart than the commonly used mercurial diuretics. In 70 trials carried out on 36 patients suffering from congestive heart failure with considerable œdema, the diuresis obtained after the subcutaneous injection of 2 ml. of thiomerin was comparable with, or greater than, that noted after the intramuscular use of equivalent amounts (0·08 g. of mercury) of mercurophylline or mersalyl-theophylline in 29 comparative studies on 21 patients. In some patients the diuresis with thiomerin was more sustained. The diuresis resulted in weight losses ranging from 2 to 17½ lb., the range of weight loss being roughly proportional to the amount of œdema present. The diuretic effect usually appeared within several hours after the injection and lasted for 24 hours or more. No systemic toxic effects were observed, and no kidney damage, as judged by urine analyses, occurred. Local reactions were absent or insignificant. S. L. W.

## BACTERIOLOGY AND CLINICAL TESTS

***p*-Aminosalicylic Acid and Salicylic Acid, Antagonism Between, Effects of, on *Mycobacterium tuberculosis*.** G. Iványovics. (*Proc. Soc. exp. Biol., N.Y.*, **70**, 462.) A freshly prepared solution of the sodium salt of *p*-aminosalicylic acid was added to a liquid medium containing 0·05 per cent. of Tween 80 and 0·5 per cent. of bovine albumin fraction. Serial dilutions by a factor of two were made. The tubes were inoculated with 0·0001 mg. (dry weight) of tubercle bacilli grown in Dubos medium and the total volume made up to 5 ml. with water. After 16 days' incubation at 37°C., 0·5 ml. of phosphate buffer, *p*H 7·0, containing 2 per cent. of formaldehyde and 0·1 per cent. of Tween 80 was added to each tube and the amount of tubercle bacilli was determined by turbidimetric readings in a Leitz universal colorimeter. Using a control culture as reference, the growth rate of bacilli in the presence of antiseptics was expressed in percentages of the full growth. Comparisons of the effect of *p*-aminosalicylic acid alone and in combination with sodium salicylate showed that the tuberculostatic action of 1 mol. of *p*-aminosalicylic acid is antagonised by 4 to 12 mols. of sodium salicylate. This effect of salicylate was shown to be highly specific and not shared by related compounds. Pantothenic acid does not inhibit the tuberculostatic effect of salicylate. E. N. I.

**Chloramphenicol: Inhibition of Bacterial Esterases.** G. N. Smith, C. S. Worrel and A. L. Swanson. (*J. Bact.*, 1949, **58**, 803.) Studies on the inhibitory action of chloramphenicol on phosphatases, nucleases and oxidising enzymes suggested that the antibiotic might act by inhibiting the metabolism of fats and related organic acid esters. Such action might involve either the hydrolysis of the fat or ester, or the utilisation of the acid and alcohol resulting from the hydrolysis. The inhibitory effect of the antibiotic was investigated on *Escherichia coli* esterase, crystalline horse

liver esterase, and the esterase activity of mitochondria. In the main compartment of a Warburg vessel were placed 0·5 ml. of 0·2 M tributyrin emulsified with 2 per cent. of acacia in 0·025 N sodium bicarbonate buffer equilibrated with carbon dioxide to give *p*H 7·40 at 38°C., 2 ml. of the sodium bicarbonate buffer and 0·5 ml. of chloramphenicol solution, the concentration varying from 0·01 μg./ml. to 3 mg./ml. One side arm contained 0·5 ml. of enzyme solution; the other contained 0·5 ml. hydrochloric acid, which was used to determine the amount of carbon dioxide retained by the solutions when testing crude enzyme preparations. With chloramphenicol concentrations in the final solution of 1 to 3 μg./ml. the enzymatic activity of living *E. coli* cells was decreased by up to 25 per cent., whereas at higher concentrations (3 to 50 μg.) there was marked stimulation of activity, the increase being from 50 to 80 per cent. Beyond 50 μg./ml. enzymatic activity was almost completely inhibited. Essentially similar results were obtained with horse liver esterase, but the inhibition at low concentrations and the stimulation at high concentrations were less marked. With mitochondria the drug inhibited only 40 to 50 per cent. of the esterase activity and there was no inhibition at high concentrations, suggesting that some barrier exists preventing reaction between the drug and the esterase within the animal cell. The effect on esterase activity of *E. coli* cells was in close agreement with the effects of the various concentrations on growth and this may provide a clue to the bacteriostatic action of the antibiotic.

H. T. B.

**Iodonium Compounds, Bactericidal Efficiency of, against Gram-negative Bacteria.** L. Gershenfeld and C. Kruse. (*Amer. J. Pharm.*, 1949, **121**, 343.) Three compounds were tested namely bis(methylphenyl)iodonium chloride (I), bis(3 : 4-dichlorophenyl)iodonium chloride (II), and bis(2 : 4-dichlorophenyl)iodonium sulphate (III), the solubility in water being for each compound about 0·1 per cent. The Gram-negative organisms employed were *Shigella alkalescens, Proteus vulgaris, Pseudomonas æruginosa, Klebsiella pneumoniæ, Salmonella enteritidis* and *Neisseria catarrhalis.* Bacteriostatic efficiencies were investigated by the U.S. Food and Drug Administration agar plate procedure and bactericidal efficiencies by the F.D.A. phenol coefficient technique using in each case dilutions of the saturated solutions. The three compounds had approximately the same bacteriostatic activities, being most effective against *Klebsiella pneumoniæ* and least effective against *Ps. æruginosa.* Compound II had the strongest bactericidal action, its effect being demonstrable in 1 : 20 dilutions of the saturated solution. Compounds I and III only showed bactericidal action when used in the saturated solutions.

H. T. B.

**Penicillin and Sulfactin : Drug-fastness Studies with *Staphylococcus aureus*.** H. E. Morton and M. J. B. Perez. (*Proc. Soc. exp. Biol., N.Y.*, 1948, **69**, 26.) Sulfactin, an antibiotic produced by a species of *Actinomyces,* resembles penicillin in being active against Gram-positive organisms. Cultures of *Staphylococcus aureus* resistant to sulfactin and others resistant to penicillin were developed *in vitro* by growing the organisms in increasing concentrations of the respective antibiotics. The sulfactin-resistant strain developed a resistance to the drug more than 1,100 times that of the original strain. Under similar conditions, the resistance of the organism to penicillin increased slightly more than 32-fold. In becoming resistant to sulfactin the strain showed no increase in its resistance to penicillin, and in becoming resistant to penicillin there was no increase in resistance to sulfactin.

H. T. B.

# PHARMACOPŒIAS AND FORMULARIES

## THE BRITISH PHARMACEUTICAL CODEX, 1949

### Its Relation to Medicine and Pharmacy in the Near East

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SINCE the beginning of this century the Arabian culture, which dominated the Near East for more than thirteen centuries, has been inevitably influenced by the French, British and, more recently, by the American cultures, and the growing influence of these varied foreign cultures makes the task of unifying the standards of medical and pharmaceutical practice a difficult one.

In Syria and Lebanon the dominating French influence was gradually replaced, in professional practice, by the American system particularly after the establishment of the American University at Beirut. Graduates of the schools of pharmacy and medicine of this University include citizens from all the countries of the Near East. On the other hand, the medical and pharmaceutical education in Iraq, Transjordan, Saudi Arabia and Palestine is closely connected with that in Egypt, which follows the British system. Other schools like the Italian, the German and the Swiss have their own groups and, everywhere in the Near East, one can find practitioners and pharmacists who advocate their own standards. Egypt, the most progressive of the Near East countries, has been in a state of uncertainty.

The varied standards adopted throughout the Near East necessitates a general dispensatory like the British Pharmaceutical Codex which, while written in English and thus meeting the demand of the graduates of the British school, still includes the principal material official in the French, the German and the United States Pharmacopœias. Moreover, it includes a considerable bulk of material concerning the crude drugs and their preparations which meet the demand of the graduates of the old school.

During the time when the British Pharmacopœia was the semi-official Pharmacopœia in Egypt, the British Pharmaceutical Codex was naturally the main reference book in pharmaceutical practice. It was one of the principal works of reference in the preparation of the first edition of the Egyptian Pharmacopœia which, according to the decision of the Second Arabian Pharmaceutical Congress will be the official Pharmacopœia of the Arabian East. There is every reason to believe that the new edition of the Codex will retain its position and become the first companion to the new Egyptian Pharmacopœia, especially as the bulk of the material in the Egyptian Pharmacopœia is a modified digest of the British Pharmacopœia, the French Codex and the United States Pharmacopœia, all of which are covered by the British Pharmaceutical Codex.

It is needless to emphasise the value of the Codex as a collection of standards; and undoubtedly, in the absence of any standards for pharmaceutical products in the Near East the Codex standards should be used. The new Codex will meet the present demand of general practitioners and pharmacists in the Near East during the transition period to the new era of unified standards to be established by the Egyptian Pharmacopœia. Until and after the publication of an International Pharmacopœia, such a cosmopolitan dispensatory as the British Pharmaceutical Codex will be of value specially in an area where more than one school of standards prevails. The new additions and modifications to the Formulary of the new Codex are welcomed in modern practice, while the sections of actions and uses should meet with great satisfaction.