

$$y = a e^{-bt} \left(\frac{1}{\sigma \sqrt{2\pi}} \int_{m_1-r}^{m_1+r} e^{-\frac{(x-m)^2}{2\sigma^2}} dx \right)$$

where: $m = m_1 - tm_2$
 $\sigma^2 = \sigma_1^2 + t\sigma_2^2$

Here, a is a constant of integration, essentially the number minted. The legal weight, as also the average of freshly minted coins is taken as m_1 , the variance at the mint as σ_1^2 . The average loss of weight per year is m_2 , and the variance of this annual loss, σ_2^2 . The legal remedy, i.e., the weight by which a coin may exceed or fall below the legal standard is called r in the formula.

When the coin is a source of metal, the first factor would account for most of the currency in circulation, particularly as the variances with modern technique of minting are very small. But with a token coin, and in any case after the passage of a greater number of years, the second factor would

begin to dominate, and the coins withdrawn rapidly from circulation by those who check the currency. The phenomenon is similar to that often seen in biology, where a gene or culture of bacteria shows exponential growth till a threshold value is reached, when the situation changes entirely, the growth makes its own surroundings lethal, and further growth is either inhibited, or the whole of the variate vanishes altogether.

¹ *Journal and Proceedings of the Asiatic Society of Bengal*, New Series, 1934, **30**, Numismatic Number.

² *Memoirs of the Archaeological Survey of India*, 1939, No. 59.

³ *Catalogue of Indian Coins in the British Museum*, Ancient India, 1930.

⁴ *Ibid.*, Andhras, W. Kṣatrapas, 1908, p. clxxvii *et seq.*

⁵ The work of A. S. Hemmy, *Journal of the Royal Asiatic Society*, 1937, pp. 1-26 must be dismissed as mere trifling with an important subject.

⁶ One coin in the 3-mark round lot should also have been so omitted, bringing the mean to 52.20, which would have fitted much better.

SULPHANILAMIDE AND DERIVATIVES IN BACTERIAL INFECTIONS

BY

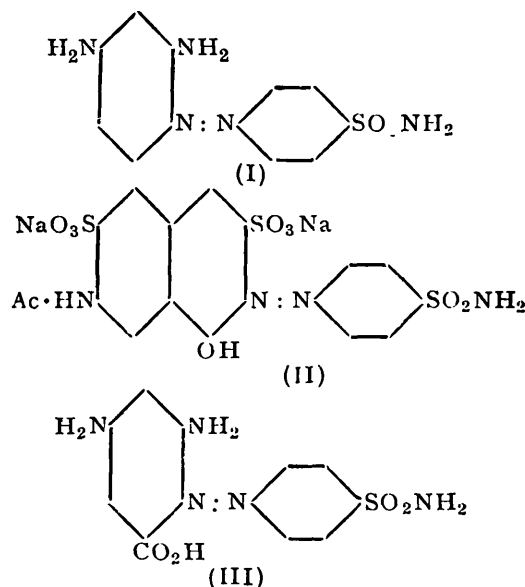
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1. PRONTOSIL AND RELATED DYES

DOMAGK'S sensational discovery¹ of the specific curative effect of 'prontosil' (I) in experimental β -hæmolytic streptococcal infections in mice, which is hailed as the "greatest discovery in modern therapeutics", appears to have been made in 1932 as a culmination of his researches dating from 1923-24 in the Elberfeld laboratories of the I. G. Farbenindustrie.² Regarding the hosts of compounds that must have been tested systematically in the course of this investigation, we are given no details. The discovery was announced on the 15th February 1935¹ only after it was confirmed by three years of clinical trials at the hands of the Rhineland practitioners, for "by untimely publication he did not want to give false hopes to doctors and patients".² This dye (prontosil) being of low solubility in water (about 0.25 per cent.), a more soluble form, "prontosil soluble" ("prontosil S", "neo-prontosil", II) was introduced (as 2.5 per

cent aqueous solution) for parenteral use, while in France, a carboxyl derivative of prontosil, "rubiazol" ("rubiazol C", III), synthesised by Gley and Girard³ came into use.



2. REDUCTION OF PRONTOSIL *in vivo*: EVOLUTION OF SULPHANILAMIDE

The next great advance in the subject was made by Tréfouël (J. and Mme.), Nitti and Bovet⁴ in November 1935. They studied systematically the antistreptococcal properties of forty-five dyes of the azobenzene group⁵ with various substituents and found that the replacement of the sulphonamide ($-\text{SO}_2\text{NH}_2$) group in prontosil (I) by $-\text{AsO}_3\text{H}_2$, $-\text{SO}_3\text{H}$, $-\text{CN}$, $-\text{CONH}_2$, $-\text{CH}_2\text{CO.CN}$ and $-\text{O.Ph}$ groups destroyed the activity, while the amino group could be replaced by other groupings without much loss of activity. This led them to formulate the important hypothesis⁴ that the therapeutic activity of prontosil is due to *para* amino-benzene-sulphonamide (sulphanilamide) liberated *in vivo* by reduction. In support of this they showed, for the first time, that the simple colourless compound, sulphanilamide (already synthesised by Gelmo in 1908 who never dreamt of its therapeutic properties), itself was as active as prontosil in experimental streptococcal infections. The above results were confirmed from various points of view by many workers. As an apparent proof of the hypothesis, Fuller⁶ actually isolated sulphanilamide from the urine of a patient treated with prontosil. Ganapathi and Rao⁷ have shown that following the feeding of six typical dyes of this group to groups of mice in therapeutic doses (10 mg.), only the therapeutically active once produce considerable blood concentrations (1.6 to 2.0 mg. per cent.) of sulphanilamide, whereas the very little active or inactive ones produce only traces.

Though till now about seventy dyes of the above group constituting various types have been reported, only a dozen of these have been found to be comparable in anti-streptococcal activity to prontosil or sulphanilamide; it is yet to be shown definitely that these active dyes possess any advantage over the parent amines. As regards both the intensity as well as the poly-valency of therapeutic effect, the dyes are inferior to the free amines. For example, sulphanilamide shows a striking therapeutic effect in (β -hæmolytic) streptococcal, meningococcal, gonococcal, bacillus welchi and *B. coli* infections; its effect in *B. typhosus* infection is considerable; in pneumococcal infections, the protection is less and in staphylococcal infections far less. The dyes show consider-

able therapeutic effect in streptococcal infections and in the rest their efficacy compared to sulphanilamide is negligible. However, both the dyes and sulphanilamide possess considerable protective effect in the virus infection, lymphogranuloma inguinale.⁸

3. SEAT OF THERAPEUTIC ACTIVITY IN SULPHANILAMIDE

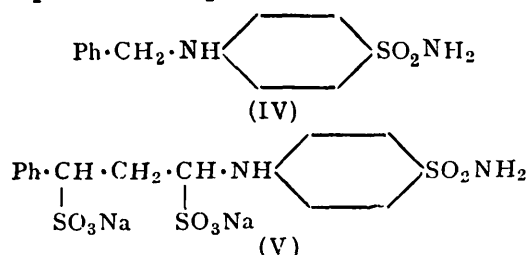
The next obvious step of elucidating the seat of chemotherapeutic activity in sulphanilamide was immediately taken up by Fourneau, Trefouels, Nitti and Bovet.⁹ They studied 130 derivatives of related structures⁵ and showed that for the antistreptococcal activity, (i) the amino and sulphonamido groups in the *para* positions of the benzene ring are necessary, (ii) the presence of an additional grouping in the benzene ring destroys the activity and (iii) the substitutions in the amino and sulphonamido radicals have variable activity depending on the nature of the substituents. These important findings led to such intense activity in the synthesis of new compounds of this group that, till now, about ninety papers have been published and forty patents taken, reporting in all about 600-700 compounds (besides a lot unpublished).

4. COMPOUNDS WITH SUBSTITUENTS IN THE AMINO RADICAL OF SULPHANILAMIDE

About 130 derivatives with various types of substituents in the amino radical of sulphanilamide have been reported, of which only about twenty possess anti-streptococcal activity comparable to that of sulphanilamide. Of these twenty derivatives, fourteen are Schiff's bases obtained by condensing sulphanilamide with variously substituted benzaldehydes.^{10,11} Of the forty-five acyl derivatives reported,^{5,12,13,14} the valeryl, caproyl,^{12,13} and pyrrolidone carboxy derivatives¹⁰ are as active as sulphanilamide. It is conceivable that all the above compounds can yield free sulphanilamide *in vivo* by hydrolysis. The guanidine¹⁵ and formaldehyde sulphoxylate derivatives¹⁶ of sulphanilamide are stated to be quite active.

4-Benzylaminobenzene sulphanamide (IV) first reported by Goissedet *et al.*,¹¹ and introduced for clinical trials (under the trade names "proseptasine", "septazine"), has been shown to be inferior to sulphanilamide in experimented infections in mice.^{10,16,17,18} It has been suggested by Lockwood and Robinson,¹⁸ though it cannot be considered to be

definitely proved, that the activity of pro-septazine is due to the sulphanilamide liberated *in vivo*. Another colourless compound, disodium *p*-(γ -phenylpropyl) aminobenzenesulphonamide- α : γ -disulphonate (V), has been introduced for clinical trials under the trade name "soluseptazine" for parenteral use. The only animal experiments reported about it by Whitby,¹⁷ do not indicate it to be superior to sulphanilamide. Though these



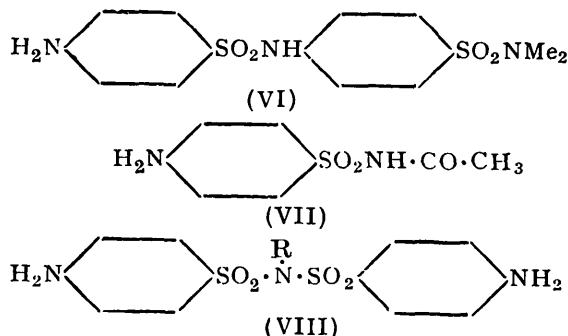
two drugs possess considerable antistreptococcal action, their therapeutic effects in other bacterial infections compared to sulphanilamide is negligible.^{16,17} Schutz¹⁹ has reported "soluseptazine" to protect rats and not mice in experimental *P. pestis* infections.

The *para* nitro, nitroso, hydroxylamino and hydrazo derivatives of benzenesulphonamide have been studied by Mayer.²⁰ The first two compounds are more active than sulphanilamide and more toxic, while the hydrazo derivative is inactive. The hydroxylamine derivative is about 100 times more bactericidal than sulphanilamide *in vitro*, but not *in vivo* (this being due to its reduction in the body to sulphanilamide). Some significance is attached to this compound in explaining the mechanism of action and also some of the toxic manifestations of sulphanilamide.^{20,21}

5. COMPOUNDS WITH SUBSTITUENTS IN THE SULPHONAMIDE RADICAL OF SULPHANILAMIDE

Forty derivatives with alkyl and aralkyl groupings substituted in the sulphonamide radical of sulphanilamide have been synthesised and tested but these have proved to be of no advantage. Of interest is the report of Adams *et al.*¹³ that five derivatives with hydroxypropyl substituents in the sulphonamide radical show striking antimeningococcal but no antistreptococcal activity. Eighty-five derivatives of 4-aminobenzenesulphonanilide with various substituents in the second benzene ring have been synthesised and tested. Those with the nitro,

amino, sulphonamido, substituted sulphonamido, N¹-sulphanilamido, sulphonic and carboxylic acid groupings in the second benzene ring are quite active and many reported to be even superior to sulphanilamide in antistreptococcal activity. Domagk reported²² *p*-aminobenzenesulphonamido benzene-*p'*-dimethylsulphonamide (known by the trade names, "Deseptal A", "Uleron", "Uliron", VI) to be superior to sulphanilamide in streptococcal and staphylococcal infections but this has not been confirmed.^{16,23} It was given a fairly extended clinical trial in gonococcal infections but has now been withdrawn due to the toxic reaction of peripheral neuritis. A series of N¹-acyl derivatives of sulphanilamide has been reported.²⁴ The acetyl derivative ("alucid", VII), though far less active than sulphanilamide in streptococcal infections, has been introduced for clinical trials in gonococcal infections with the claim that it is of low toxicity and very little of it gets acetylated *in vivo*. Crossley *et al.*²⁵ have reported five compounds of the disulphanilamide group (VIII) to be superior to sulphanilamide in antistreptococcal action.



One of these, the sodium salt of disulphanilamide (VIII, R = Na), is claimed to protect mice infected with moderate doses of the influenza virus.²⁶ Adams, Long and Johanson¹³ have reported thirty compounds with acyl substituents in the amino, and hydroxyalkyl substituents in the sulphonamido radicals of sulphanilamide. Of these, only eight are of interest for they are quite active in meningococcal but almost inactive in streptococcal infections.

6. DIPHENYLSULPHONE AND RELATED DERIVATIVES

Almost simultaneously, Buttle *et al.* in May 1937,²⁷ and Fournau, *et al.*²⁸ In June-July of the same year reported the remarkably high antistreptococcal action of 4:4'-diaminodiphenylsulphone. This diamine,

according to Buttle *et al.*²⁷ is about 100 times as active as sulphanilamide and 10 times more toxic in mice. In rabbits and monkeys, and possibly in man also, the toxicity as also the activity, do not appear to be so high. The diacetyl derivative (marked under the name "Rhodilone" in France for use particularly in gonorrhea) is relatively very little toxic and yet ten times as active as sulphanilamide. The 4:4'-dinitrodiphenyl sulphone is as active as sulphanilamide. The last two sulphone derivatives appear to be converted *in vivo* into the free amines.²⁹ The significance about these compounds is: (i) they do not contain the sulphonamide grouping and (ii) they are the best of the till then known compounds giving a definite percentage of survivors in experimental pneumococcal (type I) infections. In other types of pneumococcal infections, the action of the diacetyl derivative is not so good.³⁰ In experimental typhoid infections, it is inferior to sulphanilamide. About fifty related derivatives of this diphenylsulphone group have been tested but only the benzylidene, glucose³¹ and the formaldehyde-sulphoxylate derivatives³² of 4:4'-diaminodiphenylsulphone have been suggested to be of some advantage. However these are not in use in practical therapy to-day.

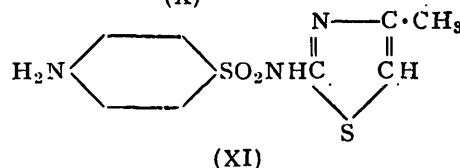
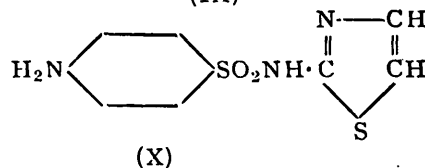
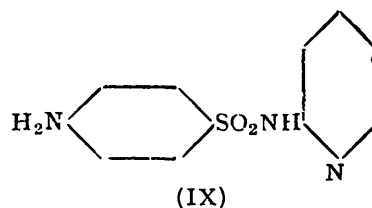
About seventy related derivatives of the diphenylsulphoxide, diphenylsulphide, diphenyldisulphide and related compounds have been studied but they are generally of far less activity. 4-Nitro-4'-amino diphenylsulphoxide has been claimed by Levaditi *et al.*³³ to possess such specific effects in gonococcal infections as the arsenicals in the spirochætal infections.

7. HETEROCYCLIC DERIVATIVES OF SULPHANILAMIDE

The discovery by Whitby,³⁴ announced in May 1938, of the remarkable protective effect of 2-N¹-sulphanilamidopyridine ("Sulphapyridine", "Dagenan", "M. & B 693," IX), one of the forty-six compounds synthesised by Ewins and Phillips of the firm, 'May and Baker', in experimental pneumococcal infections in mice, is indeed a distinct advance in the chemotherapy of bacterial infections. The "dramatic cures" obtained with it in clinical trials in cases of pneumonia, have convinced us about its remarkable therapeutic properties. This compound is more polyvalent in action than sulphanilamide. The only experimental infection in which it is shown to be inferior to

sulphanilamide is that due to *B. typhosus*; in streptococcal, meningococcal and gonococcal infections it is at least as good as sulphanilamide, while it is distinctly superior to sulphanilamide in pneumococcal, staphylococcal and *P. pestis*^{10,43} infections.

Of the hundreds of aromatic sulphur compounds synthesised and tested, though about seventy are about as active as and twenty-five distinctly superior to sulphanilamide in experimental streptococcal infections in mice, only sulphanilamidopyridine (IX) reigned supreme in its effect in pneumococcal infections. The presence of the heterocyclic ring in this compound gave thus the impetus to search for active compounds among the related heterocyclic derivatives of sulphanilamide. Thus, pyridine, quinoline, acridine, morpholine, piperidine derivatives are being tried. Though the trial cannot be said as yet to be complete, the quinoline, morpholine and piperidine derivatives do not show any promise. The search has been amply rewarded when attention was directed towards the thiazol derivatives by four groups of workers independently—by Fosbinder and Walter,³⁶ Iuda, Itikawa and So,³⁷ Lott and Bergeim³⁸ and Ganapathi and Nandi,³⁹ 2-N¹-sulphanilamidothiazol (X) and the methyl derivative (XI) have been found to be as active as sulphanilamidopyridine (IX)^{40,41,42,45} in pneumococcal infections.



Extensive researches with this thiazol derivative (X) (for which the short name "sulfathiazol" has been suggested⁴⁰) at this Institute for the past several months, have convinced us that this new drug has a great future. While being far less toxic, it is even more polyvalent in action than sulphanilamidopyridine. In experimental strepto-

coccal infections in mice it is distinctly superior to sulphanilamide.⁴² Sokhey and Dikshit⁴³ have found that it is far superior to sulphanilamidopyridine and almost a specific in experimental plague infections in mice. In staphylococcal infections also, these thiazol derivatives (X and XI) are distinctly superior to sulphanilamidopyridine. While the animal experiments are very encouraging, only extended trials have to pass the final verdict. In the meantime, similar heterocyclic derivatives are being prepared by the author and tested with the hope of obtaining even better compounds.

8. THE FUTURE OF BACTERIAL CHEMOTHERAPY

Whatever be the future of "prontosil", which has brought about a renaissance in the Chemotherapy of Bacterial Infections, Domagk has indeed earned a place next to Ehrlich. The evolution of this subject, still in the cradle, from "prontosil" to "sulfa-thiazol" indicates that we are in the right way towards the conquest of the bacterial infections. The results obtained so far give us even the optimism that some day the dream of Ehrlich of "*a therapia magna sterilisans*", will materialise at least in the treatment of some bacterial infections. There are many favourable indications for this, e.g., in experimental meningococcal and B. typhosus infections in mice, sulphanilamide can give sufficiently high protection with even one dose of the drug. The achievements so far made are by themselves no means meagre. While Ehrlich met with "one moment" of success after seven years of disappointments, in the present case, in five years have been made conquests of a variety of deadly infections due to at least five types of bacteria. With far more extended trials, we can hope to conquer even the dreaded tuberculosis and detested leprosy.

- ¹ Domagk, G., *Deut. med. Wschr.*, 1935, **61**, 250; *Angew. Chem.*, 1935, **48**, 657.
- ² —, *Zentr. Bakt. Parasit. Krankheit.*, 1939, **144**, 206.
- ³ Gley, P., and Girard, A., *Presse. Med.*, 1936, **44**, 1775; *C.R. Soc. Biol.*, 1937, **125**, 1027.
- ⁴ Tréfouël (J. and Mme.), Nitti, F., and Bovet, D. *Ibid.*, 1935, **120**, 756.
- ⁵ Idem, *Annales Inst. Pasteur*, Paris, 1937, **58**, 30.
- ⁶ Fuller, A. T., *Lancet*, 1937, **1**, 194.
- ⁷ Ganapathi, K., and Sanjeeva Rao, R., *Indian J. Med. Res.*, under publication.
- ⁸ Levaditi, C., *C.R. Soc. Biol.*, 1938, **128**, 138, 875.
- , Vaisman, A., and Reine, L., *Ibid.*, 1939, **131**, 40.
- Mac Collum and Findlay, G. M., *Lancet*, 1938, **2**, 136.
- ⁹ Fournau, E., Tréfouël (J. and Mme.), Nitti, F., and Bovet, D., *C.R. Soc. Biol.*, 1936, **122**, 258.

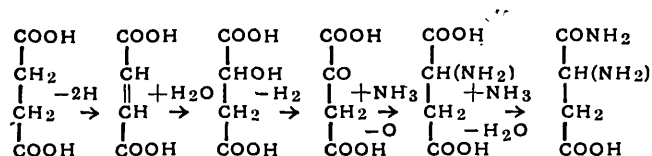
- ¹⁰ Gray, W., Buttle, G. A. H., and Stephenson, D., *Biochem. J.*, 1937, **31**, 724.
- ¹¹ Goissedet, P., Despois, R., Gailliot, O., and Mayer R., *C.R. Soc. Biol.*, 1936, **121**, 1082.
- ¹² Miller, E., Rock H. J., and Moore, M. L., *J. Amer. Chem. Soc.* 1939, **61**, 1198.
- ¹³ Adams, R., Long, P. N., and Johanson, A. J., *Ibid.*, p. 2342.
- ¹⁴ I. G. F. Patent, *C.A.*, 1938, **32**, 2958 (Indian Patent, 24441).
- ¹⁵ Buttle, G. A. H., Dewing T., Foster, G. E., Gray, W. H., Smith, S., and Stephenson, D., *Biochem. J.*, 1938, **32**, 1101.
- ¹⁶ Feinstone, W. H., Bliss, E. A., Ott, E., and Long, P. H., *Bull. John Hop. Hosp.*, 1938, **62**, 565.
- ¹⁷ Whitby, L. E. H., *Lancet*, 1937, **i**, 1517.
- ¹⁸ Lockwood, J. S., and Robinson, H. J., *J. Pharm. Exp. Ther.*, 1940, **68**, 201.
- ¹⁹ Schütz, H., *Lancet*, 1939, **1**, 266.
- ²⁰ Mayer, R. L., *Bull. L'Acad. Med.*, 1937, **117**, 727; *Biol. Med.* (Supplement), 1937, **27**, 74.
- ²¹ Schaffer, P. A., *Science*, 1939, **89**, 547; Locke, A., and Mellon, R. R., *Ibid.*, p. 231.
- ²² Domagk, G., *Klin Wschr.*, 1937, **16**, 1412.
- ²³ Mellon, R. R., Shinn, L. E., and McBroom, J., *Proc. Soc. Exp. Biol. Med.*, 1937, **37**, 563.
- ²⁴ Crossley, M. L., Northey, E. H., and Hultquist, M. H., *J. Amer. Chem. Soc.*, 1939, **61**, 2950.
- ²⁵ Idem, *Ibid.*, 1938, **60**, 2222.
- ²⁶ Clemenko, D. R., Crossley, M. L., and Northey, E. A., *J. Pharm. Exp. Ther.*, 1939, **67**, 201.
- ²⁷ Buttle, G. A. H., Stephenson, D., Smith, S., Dewing, T., and Foster, G. E., *Lancet*, 1937, **1**, 1331.
- ²⁸ Fournau, E., Tréfouël, J., Nitti, F., Bovet, D., and Tréfouël (Mme.), *C.R. Acad. Sci.*, Paris, 1937, **204**, 1763; 1937, **205**, 299.
- ²⁹ Bovet, D., Tréfouël (J. and Mme.), Nitti, F., and Hamon, M. V., *Annals. Inst. Pasteur*, Paris, 1938, **61**, 812.
- ³⁰ Cooper, F. B., Gross, P., and Lewis, M., *Amer. J. Med. Sci.*, 1938, **196**, 343; 1939, **197**, 609.
- ³¹ Buttle, G. A. H., Dewing, J., Foster, G. E., Gray, W. H. Smith, Sand Stephenson, D., *Biochem. J.*, 1938, **32**, 1101.
- ³² Bauer, H., *J. Amer. Chem. Soc.*, 1939, **61**, 617.
- and Rosenthal, S. M., *U. S. Pub. Hlth. Rep.*, 1938, **53**, 40.
- ³³ Levaditi, C., Girard, A., Vaisman, A., Ray and Richard, *C.R. Acad. Sci.*, 1937, **205**, 1018.
- ³⁴ Whitby, L. E. H., *Lancet*, 1938, **1**, 1210.
- ³⁵ Powell, H. M., and Chen, K. K., *J. Pharm. Exp. Ther.*, 1939, **67**, 69.
- ³⁶ Fosbinder, R. J., and Walter, W. A., *J. Amer. Chem. Soc.*, 1939, **61**, 2032.
- ³⁷ Iuda, K., Itikawa, Z., and So, D., *J. Pharm. Soc. Japan*, 1939, **59**, 213; *C.A.*, 1939, **33**, 820.
- ³⁸ Lott, W. A., and Bergeim, F. H., *J. Amer. Chem. Soc.*, 1939, **61**, 3593.
- ³⁹ Ganapathi, K., and Nandi B. K. *Curr. Sci.*, 1940, **9**, 67.
- ⁴⁰ Cooper, F. B., Gross, P. and Lewis, P., *Proc. Soc., Exp. Biol. Med.*, 1939, **42**, 421.
- ⁴¹ McKee, C. M., Rake, G., Greep, R. O., and Van Dyke, H. B., *Ibid.*, p. 417.
- ⁴² Ganapathi, K., *Indian J. Med. Res.*, 1940, **27**, 971.
- ⁴³ Sokhey, S. S., and Dikshit, B. B., *Curr. Sci.*, 1940, **9**, 116; *Lancet* (under publication).
- ⁴⁴ Barlow, O. W., and Homburger, E., *Proc. Soc. Exp. Biol. Med.*, 1940, **42**, 792.
- ⁴⁵ Ganapathi, K., *Proc. Ind. Acad. Sci.*, 1940, **11**, 298.

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Succinic Dehydrogenase in Germinating Seeds

ALTHOUGH asparagine occupies a central position in the amino-acid metabolism of plants little is known about the mechanism of its formation. On chemical grounds and from analogies based upon amino-acid synthesis in animal tissues it has been considered probable that the asparagine arises from succinic acid (frequently shown to be present in plants) and ammonia (arising from the deamination of amino-acids) by the following reactions (Kostytschew,¹ Chibnall²).



A serious difficulty in accepting this scheme has been the fact that succinic dehydrogenase, the enzyme necessary for catalysing the first step in the above chain of reactions, has hitherto not been found to be of general or wide distribution in the vegetable kingdom. In fact

Thunberg to whom we owe the demonstration of the existence of many plant dehydrogenases, found the occurrence of this enzyme to be limited to the seeds of *Phaseolus vulgaris*.

As the most marked accumulation of asparagine takes place in germinating seeds, we have examined the seedlings of a number of species for succinic dehydrogenase using the Thunberg Methylene Blue technique. It was found that seedlings of six species of Leguminosae tested (*Phaseolus mungo*, *Phaseolus radiatus*, *Dolichos biflorus*, *Dolichos lablab*, *Cicer arietinum*, *Vigna catjang*) all show dehydrogenase activity towards succinic acid. The enzyme was on the other hand found to be absent from the seedlings of water-melon (*Citrullus vulgaris*) and rice (*Oryza sativa*). The seedlings were ground in a granite mortar with M/15 phosphate buffer (pH 9.0), pressed through muslin and centrifuged. The supernatant liquid (which was found to contain all the activity) was used for experiment, the reaction mixture being made up of 0.5 ml. of enzyme solution, 0.5 ml.