from the pericardial sac. Buchanan and Daly (1902) give an average weight of heart as 10.5 oz. (297.7 g.) in the male and 8.5 oz. (241 g.) in the female in the English subjects. Gray (Johnston and Whillis, 1946) states 'heart continues to increase in weight and size up to an advanced period of life and this increase is more marked in men than in women'. He mentions the average weight of an adult male heart as 280 g. to 340 g. and that of an adult female as 230 g. to 280 g. Both these English authors have met with higher weight incidence in their adult subjects as compared with our findings. No authentic Indian figures have been published so far. Mody (1947) has calculated weights of organs from dead bodies on which a post-mortem examination was held by him in the United Provinces. However, he has taken bodies of a very diverse age group varying from 10 to 70 years for his averages. Moreover, the exact number in the series is not mentioned and statistical calculations on the mean, etc., are not on record. It is possible that some of the figures refer to medicolegal autopsies in which dead bodies were brought from distances, and as pointed out in our previous paper, the state of decomposition would materially interfere in the calculation of the weights. Mody (1947) mentions the average heart-weight in the male as 10 oz. (28.3 g.) and in the female as 6.5 oz. (184.3 g.). It is presumed that the observations refer to Indian subjects exclusively.

Heart is an organ which can increase in weight in response to work load. It is evident, therefore, that these figures would not be applicable to soldiers, aviators and athletes, in a perfect normal healthy condition.

Turning to another aspect of the calculations, instead of inferring weight of the heart from the mean of actual weights in the series, a better proposition would be to establish the ratio that the weight of the heart holds to the total body-weight. This will do away with vagaries arising out of significant differences in the body-weight. With this end in view, the ratios of heart-weights to body-weights were calculated from data of 47 males (out of the gross collection of 109 and including the 28 referred to in the preceding paragraphs).

Ranges of ratios are from 142 to 291.09 and the mean of the ratios is 162.78 with S.D. of 30.86 and co-efficient of variation of 17.21.

All these figures obtained by us are lower than those obtained by European workers. Our calculations do not by any means represent a cross section of the Indian population but they are a result of a planned study of a limited group from autopsies in Bombay over a fairly long period.

Summary

Heart-weight, body-weight and ratios of heart-weight to body-weight are presented. Statistical mean C.V. and S.D. for ratios of the heart-weight to the body-weight and for the heart-weight separately calculated for males in age group 18 to 30 years are presented.

REFERENCES

BUCHANAN, W. J., and DALY, F. J. (1902). *Indian Med. Gaz.*, **37**, 56.

GHARPURE, P. V., and JHALA, H. I. (1949). *Ibid.*, **84**, 192.

JOHNSTON, T. B., and WHILLIS, J. (1946). *Gray's Anatomy*. Longmans Green and Co., London.

MODY, J. P. (1947) .. *A Textbook of Medical Jurisprudence and Toxicology*. N. M. Tripathi Ltd., Bombay.

BIBLIOGRAPHY

JONES, F. W. (1946) .. *Buchanan's Manual of Anatomy*. Baillière, Tindall and Cox, London.

EFFECT OF SULPHADIMETHYLPYRIMIDINE ON THE MICROBIAL SYNTHESIS OF THIAMINE AND NICOTINIC ACID IN HUMAN INTESTINE

By H. N. DE
M. C. MALAKER
and
A. K. PAUL

(*Nutrition Research Unit, I. R. F. A., Biochemical Laboratory, Dacca University, East Pakistan*)

Introduction

IN recent years it has been realized that the micro-organisms of the intestine possess the capacity to synthesize a large amount of some essential vitamins which are absorbed and utilized by the host animal and that bacteriostatic agents like sulphonamides can effectively inhibit this intestinal synthesis of the vitamins. With these drugs as new tools and with improved technique of estimation, it has been shown by some nutrition experts and bacteriologists that rats can synthesize some members of B-complex, vitamins K and E in the intestine. Najar *et al.* (1943, 1944), Ellinger *et al.* (1944, 1945), Ellinger and Coulson (1944) and Oppel (1942) by conducting experiments on human subjects have reported that similar synthesis of thiamine, riboflavin, nicotinic acid and biotin takes place also in the human intestine. They have further shown that insoluble sulphonamides as sulphaguanidine and sulphasuxidine, because of their poor absorption through the intestine, induce maximum inhibition in the biosynthesis of the above vitamins of the B group. Kornberg *et al.* (1944) in course of their study of the biosynthesis of vitamin K succeeded in producing the deficiency of this vitamin in rats by using soluble sulphonamides as sulphapyrazine, sulphadiazine and sulphathiazole. By comparative study with other insoluble sulphonamides

they have found the order of effectiveness as follows : sulphapyrazine most effective, sulphadiazine and sulphathiazole next and sulphanilamide, sulphasuxidine and sulphaguanidine least effective. From the survey of the above works it is evident that the insoluble sulphaguanidine and sulphasuxidine which produce marked inhibition on the microbial synthesis of thiamine, nicotinic acid and riboflavin fail to produce any distinct effect on the biosynthesis of vitamin K in the intestine. It, therefore, appears that the inhibition of the synthesis of any particular vitamin in the intestine due to any sulphonamide is due to the specificity of the bacteriostatic action of the sulphonamides towards the different intestinal micro-organisms that take part in the synthesis of the vitamins. Investigation has, therefore, been begun in this laboratory to study how the different sulphonamides available in the market behave in inhibiting the biosynthesis of thiamine, nicotinic acid and other members of B-complex and also to study the actual mechanism of inhibition.

For systematic survey, the sulphonamides so far prepared may be classed under three major groups :—

(*A*) *Insoluble in water, acid and alkali.*—Sulphaguanidine, succinylsulphathiazole and phthalylsulphathiazole.

(*B*) *Soluble in acid and alkali but sparingly soluble in water.*—Sulphadiazine and sulphadimethylpyrimidine.

(*C*) *Soluble in water, acid and alkali.*—Sulphanilamide, sulphathiazole, sulphapyridine and sulphapyrizine.

Since much work had been done with the sulphonamides of group A, our attention was directed to the study of those of groups B and C and in the present communication, which represents the first of the series of works begun here, sulphadimethylpyrimidine—a typical member of the sulphonamides of group B—has been selected to investigate its effect on the microbial synthesis of thiamine and nicotinic acid in the human intestine and also to see whether the application of this drug interferes with the absorption and storage of the above vitamins.

Experimental

Experiments were carried out on five adult human subjects of ages varying from 25 to 40 years and of weights varying from 45 to 53 kilos. They were kept under proper care and strict observation in the hospital and were given daily the weighed amount of the basal diet for the whole of the experimental period which lasted from eighteen to twenty-four days.

In experiment no. 1 the subjects were first fed on the basal diet and after a preliminary period of three days the 24 hours' urine was collected for a further period of three consecutive days on that basal diet and their thiamine contents were measured. After this the basal diet was supplemented with daily dose of 3 gm. sulphadimethylpyrimidine ('sulphamezathine' of Imperial Chemical Pharmaceutical, Ltd.) in three equal doses (each dose two tablets of 0.5 gm.) after an initial dose of 3 gm. The urine on this supplement was collected for a period of three days after a preliminary period of same days on this drug. To see whether the above sulpha drug supplement interfered with the absorption and storage of thiamine, a test dose of 5 mg. of thiamine was then given along with the above drug and the urine on such double supplements was collected for a period of three consecutive days after same preliminary period as before. The recovery of the test dose of the above vitamin under the influence of the above sulpha drug was then compared with that when the test dose was given alone with the basal diet without the sulpha drug supplement as carried out in experiment no. 3. The influence of the above sulphadimethylpyrimidine on the biosynthesis of nicotinic acid has been studied in another set of experiment no. 2 and this was carried out in a manner similar to that adopted in experiment no. 1 except that in this case the test dose used was 300 mg. nicotinic acid and the recovery of this test dose with sulpha drug was compared with that when it was used without sulpha drug as in experiment no. 4.

Thiamine and nicotinic acid of the urine were measured according to the following methods :

Thiamine.—This was estimated by the base exchange technique using 'Decalso'* as selective absorbent according to the method of Hennessy and Cerecedo (1939) as modified by Brown *et al.* (1943). Decalso was used for adsorption and purification of thiamine.

Nicotinic acid.—This was estimated after hydrolysis with acid, and with alkali and urea according to the method of Swaminathan (1942) with some alterations based on the technique of Wang and Kodicek (1943) and the values represent nicotinic acid, nicotinamide and nicotinuric acid, and also trigonelline in which N-methyl-nicotinamide—an important product of nicotinic acid metabolism in urine—is also included and all these have been expressed as total nicotinic acid.

The chronological order of the experimental periods is denoted by the arithmetical numbers as P-II, P-IV, etc., and the periods which do not figure in the tables must be assumed to be the preliminary periods for the succeeding ones. The results of the above four experiments have been summarized and presented in the table.

Results

Effect of sulphadimethylpyrimidine on the biosynthesis of thiamine.—It is observed from the results of experiment no. 1 that the urinary

* Decalso F used for the estimation of thiamine was received from Messrs. Permutit Co., Ltd., England, to whom our thanks are due.

TABLE

Effect of sulphadimethylpyrimidine on thiamine and nicotinic acid excretion in five normal human subjects. The figures indicate the daily average values of an individual. Basal diet composed of 550 to 600 gm. rice, 70 gm. pulse, 70 gm. fish, 200 gm. vegetables, 30 gm. mustard oil, and 4 oz. milk twice a week

	Period	Diet and supplement		Urinary elimination	
				Range	Mean
Experiment no. 1. Experiment with sulpha drug and sulpha drug + test dose of thiamine.	P-II	Basal diet	Total B_1 in µg. per day	98.0 –138.0	120.0
	P-IV	Basal diet + 3 gm. sulpha drug.	Do.	18.0 – 32.0	25.0
			Per cent reduction of elimination due to sulpha drug.	73.3 – 84.2	78.8
	P-VI	Basal diet + 3 gm. sulpha drug + 5 mg. test dose of thiamine.	Total B_1 in µg. per day	643.0 –838.0	725.0
			Per cent recovery of the test dose.	12.3 – 16.1	14.0
Experiment no. 2. Experiment with sulpha drug and sulpha drug + test dose of nicotinic acid.	P-II	Basal diet	Total N.A. in mg. per day.	6.09– 8.33	6.95
	P-IV	Basal diet + 3 gm. sulpha drug.	Do.	11.34– 14.91	12.98
			Per cent reduction of elimination due to sulpha drug.	72.3 –100.2	86.9
	P-VI	Basal diet + 3 gm. sulpha drug + 300 mg. test dose of nicotinic acid.	Total N.A. in mg. per day.	71.86– 82.21	76.72
			Per cent recovery of the test dose.	19.1 – 24.0	21.2
Experiment no. 3. Test dose experiment with thiamine.	P-II	Basal diet	Total B_1 in µg. per day	102.0 –132.0	115.0
	P-IV	Basal diet + 5 mg. thiamine as test dose.	Do.	733.0 –919.0	877.0
			Per cent recovery of the test dose.	12.4 – 17.2	15.2
Experiment no. 4. Test dose experiment with nicotinic acid.	P-II	Basal diet	Total N.A. in mg. per day.	6.18– 7.24	6.42
	P-IV	Basal diet + 300 mg. nicotinic acid as test dose.	Do.	62.77– 78.53	72.60
			Per cent recovery of the test dose.	18.5 – 24.1	22.0

The subjects employed were : B. S.—body-weight 49 kilos, age 29 years.
M. R.— „ 48 „ „ 32 „
A. D.— „ 45 „ „ 25 „
B. M.— „ 53 „ „ 40 „
S. T.— „ 46 „ „ 30 „

thiamine excretion of the five subjects on the basal diet ranged from 98 to 138 µg. with the mean value of 120 µg. After oral administration of 3 gm. of sulphadimethylpyrimidine for six days the daily excretion of the above vitamin in the urine decreased to the limit of 18 to 32 µg. with the mean value of 25 µg. The average per cent reduction in the elimination of thiamine under the influence of the above sulphonamides was found to be 78.8 when the subjects were then given a test dose of 5 mg. of thiamine along with the above sulpha drug in the succeeding periods P-V and P-VI so as to determine as to whether the ingestion of the drug in the previous periods along with the basal diet only affected the absorption and storage of thiamine; it was found that 12.3 to 16.1 per cent of the ingested test dose was recovered in the urine and these values almost corresponded to those obtained in another experiment no. 3 after ingestion of similar test dose of thiamine without the above sulphonamide supplement. That the above sulpha drug also does not interfere with the estimation of thiamine by fluorimetric method is evident from the fact that the thiamine contents of the samples of normal urine did not show any variation even when the sulphonamide was added to them.

All the above observations clearly indicate that the reduction of thiamine excretion as obtained after oral administration of sulphadimethylpyrimidine in period IV of experiment no. 1 is not due to interference with the absorption and storage of thiamine but may be ascribed as due to decrease in the biosynthesis of this vitamin in the intestine resulting from the inhibition of the growth of those species of micro-organisms which lead to the synthesis of a large amount of this vitamin in normal conditions.

Effect on nicotinic acid synthesis.—In experiment no. 2 it has been found that the subjects on basal diet excreted from 6.09 to 8.33 mg. or with the mean value of 6.95 mg. of nicotinic acid daily in the urine. Unlike the previous experiment, the oral administration of sulphadimethylpyrimidine in daily dose of 3 gm. in this case led to the increase of the urinary elimination of nicotinic acid to an enormous extent—the range being 11.34 to 14.91 mg. with the mean value of 12.98 mg. per day. The percentage increase of the nicotinic acid excretion due to administration of the above sulphonamide was found to vary from 72.3 to 100.2 with the mean value of 86.9. When the test dose of 300 mg. nicotinic acid was given to the subjects along with this sulphonamide the percentage recovery of the added test dose was found to lie between 19.1 and 24.0 with the mean value of 21.2 and this value corresponds with that obtained in experiment no. 4 by administering similar test dose without any sulphonamide. This shows clearly that the oral administration of sulphadimethylpyrimidine does not interfere with the absorption and utilization of nicotinic acid in the body. It has further been observed that the addition of this sulphonamide to the urine does not affect the colour reaction in the estimation of nicotinic acid by the cyanogen bromide reagent. So it seems evident that the increased elimination of nicotinic acid after oral administration of the above sulphadimethylpyrimidine is due to increase in the availability of the total nicotinic acid synthesized by the intestinal flora.

It is suggested that under normal conditions a competition exists between the microflora which synthesize and those which destroy or utilize nicotinic acid and other vitamins for their growth. Most probably the above sulphonamide inhibits the growth of the latter species of micro-organisms of the intestine and thus releases a fair amount of nicotinic acid, which would otherwise have been utilized by these micro-organisms for their metabolic activities, for absorption through the intestine and for utilization within the body of the human subject.

The results presented in the table also give some indication as to the total amount of nicotinic acid synthesized daily in the human intestine. Fifty to 80 per cent reduction in the urinary elimination of nicotinic acid—which amounts to about 3 to 4 mg.—due to oral ingestion of sulphaguanidine and sulphasuxidine as observed by Najar *et al.* (*loc. cit.*) and Ellinger *et al.* (*loc. cit.*) represents only a portion of the total synthesized acid left unutilized by other species of intestinal micro-organisms. From the results of the present investigation it is observed that after oral administration of sulphadimethylpyrimidine to the adult human subjects there is an increase in the elimination of nicotinic acid by about 6 mg. and this value represents the amount of the synthesized acid which is destroyed or utilized by some micro-organisms in the intestine in the normal conditions. So the minimum quantity of nicotinic acid synthesized daily in the human intestine is between 9 to 10 mg.

Discussion

Micro-organisms which take part in the synthesis of vitamin B_1 do not require it as a growth factor in their medium and the vitamin B_1 synthesized by them functions as an essential metabolite taking part in the various enzymatic reactions required for their growth. Since both thiamine and sulphadimethylpyrimidine, used in the present investigation, contain a common pyrimidine ring it seems that the decrease in the elimination of thiamine after oral dose of the above sulphonamide is probably due to inhibition of the growth of the thiamine synthesizing intestinal microflora as a result of diffusion of this sulphonamide within their cells and of interference with the essential enzymatic reactions by competition with the enzyme constructed from vitamin B_1. The competition with another essential metabolite p-amino-benzoic acid as postulated by Fildes (1940) may also be partly responsible for the above inhibition of the growth of thiamine synthesizing bacteria.

The increased nicotinic acid elimination observed after oral administration of the above sulphonamide may be explained in the following ways :

1. It is not unlikely that the micro-organisms which synthesize thiamine require for their growth nicotinic acid which is available from the large store synthesized by other species. As an example it may be cited that propionic and lactic acid bacteria (some species and strains), *dysentery bacilli* and *C. diphtheriæ* are able to synthesize thiamine but require nicotinic acid as the growth factor in their medium. So in the present investigation when the sulphadimethylpyrimidine is administered the growth of the thiamine-synthesizing micro-organisms is inhibited and large amount of nicotinic acid required for their metabolic activities is thus released for absorption and utilization by the human body.

2. Other species of micro-organisms not synthesizing thiamine may also be present in the intestine which require in their medium, in addition to nicotinic acid, also vitamin B_1 which is available both from the endogenous source, *i.e.*

from the micro-organisms which synthesize this in the intestine and also from the exogenous dietary source. When the above sulphonamide is administered the endogenous source is stopped as a result of inhibition of the growth of the vitamin B_1 synthesizing micro-organisms according to the mechanisms discussed above and the exogenous dietary source seems to be blocked extracellularly by the drug and thus when all the available sources of thiamine are stopped, the growth of the above species of organisms is inhibited and a large amount of nicotinic acid is thereafter released for absorption and utilization by the human body. Similar blocking has also been suggested by Knight (1945) in explaining the inhibition of the growth of *Lactobacillus arabinosus* (requiring nicotinic acid for growth) after addition of sulphapyridine in the medium as observed by Tepley *et al.* (1943).

From the above discussion it is evident that the sulphadimethylpyrimidine although highly soluble in acid and alkali exerts its bacteriostatic action on those micro-organisms which synthesize thiamine and which utilize or destroy nicotinic acid in the intestine. It may, therefore, be postulated that the capacity of any particular sulphonamide inhibiting the microbial synthesis of the vitamins in the intestine depends not only on the rate of absorption through the intestine but also on the specificity of the bacteriostatic action of the drug towards the micro-organisms involved in the process of biosynthesis of the vitamins. This antibacterial action is dependent on its structural relationship with the growth factor or the essential metabolite of the different micro-organisms that take part in the biosynthesis.

Summary

1. Oral administration of sulphadimethylpyrimidine to five adult human subjects decreased the average urinary elimination of thiamine from 120 to 25 μg. The average percentage reduction was found to be 78.8.

2. In case of nicotinic acid the average urinary elimination was found to increase from 6.95 mg. to 12.98 mg. and the average percentage increase was calculated to be 86.9.

3. The recovery of the test dose of thiamine and nicotinic acid administered with and without the above sulphonamide supplement has revealed that this drug does not interfere with the absorption and utilization of the above vitamins.

4. The decreased elimination of thiamine and increased elimination of nicotinic acid due to ingestion of the above sulphonamide are probably due to inhibition of the growth of those micro-organisms which synthesize thiamine and those which utilize or destroy nicotinic acid and the mechanism of the inhibition has been discussed.

5. From the data obtained in the present investigation the minimum quantity of nicotinic acid synthesized in the human intestine has been calculated to be 9 to 10 mg.

REFERENCES

BROWN, E. B., HAMM, J. C., and HARRISON, H. E. (1943). *J. Biol. Chem.*, **151**, 153.

ELLINGER, P., BENESCH, R., and KAY, W. W. (1945). *Lancet, i*, 432.

ELLINGER, P., and COULSON, R. A. (1944). *Biochem. J.*, **38**, 265.

ELLINGER, P., COULSON, R. A., and BENESCH, R. (1944). *Nature*, **154**, 270.

FILDES, P. (1940) .. *Lancet, i*, 955.

HENNESSY, D. J., and CERECEDO, L. R. (1939). *J. Amer. Chem. Soc.*, **61**, 179.

KNIGHT, B. C. J. G. (1945). *Vitamins and Hormones*, **3**, 196. Academic Press, New York.

KORNBERG, A., DAFT, F. S., and SEBRELL, W. H. (1944). *Pub. Health Rep.*, **59**, 832.

NAJAR, V. A., and HOLT, L. E. (1943). *J. Amer. Med. Assoc.*, **123**, 683.

NAJAR, V. A., JOHNS, G. A., MEDIARY, G. C., FLEISCHMANN, G., and HOLT, L. E. (1944). *Ibid.*, **126**, 357.

OPPEL, T. W. (1942) .. *J. Clin. Invest.*, **21**, 630.

SWAMINATHAN, M. (1942). *Indian J. Med. Res.*, **30**, 537.

TEPLEY, L. J., AXELROD, A. E., and ELVEHJEM, C. A. (1943). *J. Pharmacol. and Exper. Therap.*, **77**, 207.

WANG, Y. L., and KODICEK, E. (1943). *Biochem. J.*, **37**, 530.

CLINICAL HYDROPHOBIA WITHOUT CONTACT WITH RABIES-TRANSMITTING ANIMAL

By P. L. DESHMUKH, M.D., D.T.M. & H., F.C.P.S.

Hon. Physician, Sassoon Hospitals, Poona, Hon. Lecturer in Medicine, B. J. Medical College, Poona

IT is universally believed that clinical hydrophobia occurs in man only after coming in contact with a rabid animal. The virus of rabies is transferred by the saliva of such an animal to a raw surface on the human body and clinical symptoms of hydrophobia may appear after a varying incubation period of about three months, though in some instances, the latter period may be greatly prolonged. Two cases reported below and three others seen in private practice have tended to shake the writer's belief in the accepted method of the transmission of the virus by the bite or licking by an infected animal as the only mode of infection, and have set him thinking whether there may not be some other mode of transference of the virus.