is apparently 50 % regeneration; (b) $\lambda_{\max}$ is shifted about 8 m μ . towards shorter wavelengths.

2. This result has been obtained with frog, rat and ox rhodopsin, and the amount of regeneration is, within the experimental error, 50 %.

3. A working hypothesis concerning the structure

of the rhodopsin chromophore and the mechanism of its photodecomposition is presented.

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Effect of Sulphonamides on the Aneurin Economy in Animals

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It is well established that many of the toxic effects of sulphonamides on animals fed purified diets are caused by inhibition of bacterial synthesis of some of the B vitamins in the intestine, resulting in a definite syndrome due to the deficiency of these vitamins. Few attempts have been made, however, to determine if the sulphonamides have a more direct effect on the metabolism of the B vitamins in the animal body. In a preliminary report (Slater, 1946), it was shown that sulphadiazine caused an alteration of the aneurin economy in the animal (human and rat), and that this effect was apparently unrelated to bacterial synthesis. Further studies of this effect have now been made, with the primary object of determining whether it possessed any similarity to the action of sulphonamides on micro-organisms, e.g. whether it was reversed by *p*-aminobenzoic acid (PAB).

METHODS

Animals and cages. Hooded rats of an inbred strain were used. They were weaned at 3 weeks and were usually 4–5 weeks old at the start of the experiment. Different groups were carefully matched with regard to litter, sex and body

weight. The rats were housed in individual cages with wire-mesh floors. The food consumption was measured to the nearest 0.05 g. Water was provided *ad lib*.

Diets. One of the aims of this investigation was to determine if the effect of sulphonamides on animal tissues was reversed by PAB. It was necessary, therefore, to use a diet which did not contain any PAB. At the time when this work was started (1944–5), it was impossible to obtain sufficient pure folic acid and biotin to prepare a purified diet, essentially free from PAB, containing these two vitamins. Thus the basal diet used in the early experiments was deficient, not only in PAB, but also in folic acid and biotin. Rats fed such a diet without any sulphonamide do not develop biotin or folic acid deficiency, since the two vitamins are normally provided in adequate amounts by bacterial synthesis in the intestine. The addition of sulphonamides to the diet, however, prevents the growth of the bacteria responsible for this synthesis and, consequently, precipitates a folic acid and biotin deficiency. Later, when it was shown that PAB did not reverse the effect of sulphadiazine, it was possible to use a basal diet containing both PAB and sufficient dried liver to supply all the folic acid and probably the biotin required by the rat. It was found that 4% dried ox-liver powder in the diet was sufficient to prevent the blood dyscrasia which occurred when sulphonamides were incorporated in the diet lacking the liver powder. Thus two different diets, *A* (deficient in folic acid, biotin and PAB) and *B* (containing these vitamins) were employed in these experiments. The vitamins whose metabolism was being studied were fed daily to each rat by pipette. The other vitamins were incorporated in the food. The composition of the two diets is given in Table 1.

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Table 1. *Composition of basal diets*

	Amount provided by 100 g. (dry wt.)	
	Diet A	Diet B
Casein (Labco vitamin-free) (g.)	18	18
Sucrose (g.)	73	68
Salt mixture* (g.)	4	4
Dried whole ox liver† (g.)	—	4
Peanut oil (g.)	2.5	3.1
Cod liver oil (g.)	2	2.5
Wheat germ oil (g.)	0.35	0.43
2-Methyl-1:4-naphthoquinone (mg.)	5	6
Nicotinic acid (mg.)	5	2.5
Pyridoxin (mg.)	0.5	0.25
Inositol (mg.)	16	8
Calcium pantothenate (mg.)	4	2
Choline chloride (mg.)	200	100
p-Aminobenzoic acid (mg.)	—	10
Riboflavin (mg.)	—	0.33

* Hubbell, Mendel & Wakeman (1937).

† Two preparations of dried ox liver were used. The first, used in experiments described in Table 4, provided 17 μ g. aneurin/100 g. dry wt. of diet; the second, used in the other experiments, provided 12 μ g. aneurin/100 g. dry wt. of diet.

The drugs were incorporated in the food. The percentage of drug in the diet is calculated on a dry weight basis. The amount of drug fed was insufficient to cause gross damage to the kidney or anuria due to precipitation of the drug or its acetyl derivative in the tubules of the kidney, except in one particular case which is discussed below.

Conduct of experiments. It is common in experiments of this type to use the 'paired feeding' technique, i.e. to keep the food consumption of the animals with the greater appetite down to that of those with the smallest appetite, in order to separate specific effects from those due to inanition. Except where otherwise stated, a slight variation of this procedure was followed in the present experiments, in order to allow for possible differences in the efficiency of food utilization. The food consumption of each rat was arranged so that rats from the same litter, which comprised one rat from each group, grew at the same rate as the slowest growing member of the litter, when fed *ad lib*.

Aneurin content of livers. Immediately after removal from the animal, the liver was weighed and treated with 35 ml. acetate buffer, pH 4.5, in a Waring blender for 2 min. Takadiastase (0.1 g.) and papain (0.1 g.) were added, and the treatment continued for 1 min. The mixture was transferred to a stoppered bottle, 5 ml. toluene added and the whole incubated at 37° for 18 hr. The resultant digests were poured through glass wool into a 100 ml. standard flask, and the precipitate washed once with 2 ml. 5N-H₂SO₄ and several times with water, the filtrate being finally made up to 100 ml. The aneurin content of this solution remained unchanged after several weeks at 4°.

The aneurin content was determined by the modification of Jansen's (1936) thiochrome method which has been used in this laboratory for several years, but which has not hitherto been described. A detailed description follows, since the specificity of the method is relevant to the subject of this paper. A 20 ml. sample of the extract was pipetted into a 50 ml. glass-stoppered measuring cylinder and 20 ml. into a flask containing 1 ml. of an aneurin solution (5 μ g./ml.). After mixing, 20 ml. of the latter were pipetted into a

second measuring cylinder. 20 ml. *n*-Butanol (redistilled and then saturated with water) were added to each cylinder, the contents shaken for 2 min. and then the layers allowed to separate. The two aqueous layers were called X and Y respectively. Four measuring cylinders received the following solutions, in the order shown, mixing well after each addition.

Reagents	Cylinder no.			
	1	2	3	4
	(ml. reagents used)			
Methanol (redistilled)	4	4	4	4
7.5N-NaOH	2	2	2	2
Water	0.3	—	0.3	—
5% (w/v) K ₃ Fe (CN) ₆	—	0.3	—	0.3
Solution X	4	4	—	—
Solution Y	—	—	4	4
<i>n</i> -Butanol (saturated with water)	20	20	20	20

The ferricyanide was added just before the solution X or Y. Exactly 1.5 min. was allowed for the reaction before adding the butanol. Immediately after adding the butanol, the mixture was shaken for 2 min. and poured into a separating funnel. After separation (usually light centrifugation was necessary), the aqueous layer was run off. The measuring cylinder was then rinsed with 4 ml. water which was added to the contents of the separating funnel and the mixture shaken for 1 min. After light centrifugation, if necessary, the aqueous layer was run off and the butanol layer cleared with 2 ml. ethanol. The solution was kept in the dark until its fluorescence was measured (within 3 hr. of preparation) in a Klett photoelectric fluorimeter (Slater & Morell, 1948).

From the four readings in the fluorimeter, the aneurin content of the extract was calculated from the expression, aneurin content of extract

$$= \frac{0.25(R_2 - R_1)}{1.05(R_4 - R_3) - (R_2 - R_1)} \mu\text{g./ml.},$$

where R_1 , R_2 , etc. represent the fluorimeter readings from the four cylinders.

With several different types of materials analysed in this laboratory it has been found essential to use the 'internal standard' procedure, since the conversion of aneurin to thiochrome is not quantitative and may differ considerably in different materials. For the same reason, it is necessary to standardize rigorously the procedure in order to obtain reproducible readings, and duplicate measurements are desirable. Separate 'blank' measurements in the two solutions X and Y are necessary, since it was found that shaking an alkaline solution of aneurin with air, under the conditions employed in preparing the 'blank' solution, causes the oxidation of about 2.5% of the aneurin to thiochrome.

Allowance was made for the aneurin content of the takadiastase and papain, which was determined separately. In a separate control experiment, a rat liver was halved. A known amount of pure aneurin was added to one half and the aneurin contents of both halves determined as above. The recovery of added aneurin was 92% of the theoretical. The standard error of a single determination by this method was 3.7% of the mean.

The specificity of this method for rat liver was tested on the extract of a liver of a rat fed a very low aneurin diet by destroying the aneurin, both in the extract itself and in the

extract to which aneurin was added, by treatment with 2% (w/v) Na_2SO_3 at pH 5 and 100° for 30 min. (cf. Schultz, Atkin, Frey & Williams, 1941; Slater & Rial, 1942). After this treatment, all four readings (R_1 – R_4) were about the same. Thus, if there is any substance in the rat-liver extracts which interferes with the aneurin determination by contributing to the value obtained, it must be similar to aneurin in the following respects: (1) not extracted by butanol from an acid aqueous solution; (2) converted by alkaline ferricyanide to a blue-fluorescent compound which is extractable by butanol from alkaline aqueous solution (even if the substance is, itself, blue-fluorescent and is extractable by butanol from alkaline aqueous solution, it will not affect the determination unless the fluorescence is altered by oxidation); (3) any fluorescent compound formed in this way must be activated by light of approximately the same wavelength as for thiochrome and its fluorescence spectrum must be similar (to the eye, the fluorescence of the solution prepared from the liver extract was similar to that of a solution of thiochrome); (4) it must be destroyed by sulphite to give compounds not interfering in the thiochrome test. It is considered that it is very unlikely that the extracts will contain a substance resembling aneurin in all these respects, and that the specificity of the thiochrome method compares favourably with that of most biochemical analyses.

Sulphadiazine was not itself fluorescent when tested in a concentration (1%) much greater than that found *in vivo*, nor did it give a fluorescent compound on oxidation.

Aneurin content of carcass of rat after removal of liver. The rat was killed by decapitation and the blood allowed to drain into 100 ml. acetate buffer, pH 4.5. The carcass was skinned and the liver and kidneys removed and weighed, the liver being treated as above and the kidneys added to the acetate buffer. The gastro-intestinal tract was also removed, flushed out with water and then added to the buffer solution. The remainder of the carcass, including the head, was cut up with scissors, added to the buffer, and the whole treated in a Waring blender for 2 min. Takadiastase (1 g.) and papain (1 g.) were added and the treatment continued for 1 min. The skin and fur were cut up and added to the mixture which was then treated in the blender for a further 2 min. Toluene (20 ml.) was added and the mixture incubated at 37° for 24 hr. H_2SO_4 (20 ml. of 5N) was then added and the mixture filtered through glass wool. The precipitate was washed five times with water and the filtrate allowed to settle in a separating funnel. The aqueous layer was separated from the toluene layer and diluted to 500 ml. The aneurin content of the extract was determined as above. The sulphite procedure on both control and sulphadiazine-fed rats showed that the thiochrome method was specific when applied to the rat carcasses.

Riboflavin contents of the same extracts were determined by the method of Slater & Morell (1946).

Pyruvate content of blood. In order to obtain the 'resting value' of the pyruvate in the blood, the animal's movements were restricted for some hours before killing by placing it in a closely fitting tube with wire mesh at the ends. For the same reason, the animals were usually fasted overnight before killing by decapitation. The blood was collected into a weighed 10 ml. stoppered centrifuge tube containing 4 ml. of 0.7N ice-cold trichloroacetic acid. The tube was shaken, reweighed, allowed to stand 20 min. at 0° and centrifuged. A 2 ml. sample of the supernatant was used for the pyruvate determination and the remainder for sulphadiazine de-

termination. Pyruvate was determined by the 'method A' procedure of Friedemann & Haugen (1943). The colour was measured in a Spekker absorptiometer, using an Ilford 601 filter and the concentration of pyruvate was calculated from a standard curve obtained at the same time with Na pyruvate (98.5% pure), prepared from crude pyruvic acid by the method of Lardy (1945). This method is not specific for pyruvate, but estimates other ketonic acids, e.g. α -ketoglutarate. Sulphadiazine did not give a colour in this reaction.

Sulphadiazine (free + acetylated) was measured in the trichloroacetic acid extract by Fantl's (1940) method.

Statistics. The results were treated by the analysis of variance technique as described by Goulden (1939).

RESULTS

Effect of aneurin intake on aneurin content of liver.

For purposes of comparison with the effect of sulphonamides, a preliminary experiment to determine the effect of changes in the aneurin intake of the rats, in the absence of sulphonamides, was carried out, with results shown in Fig. 1. There is an approximately linear relationship between the aneurin content of liver and the dietary intake over the range 7.6–20.7 $\mu\text{g./day}$. Apparently 20.7 $\mu\text{g./day}$ is not sufficient to saturate the tissues in young rats, since the curve shows no signs of reaching a maximum.

Fig. 1. Effect of different dietary intakes of aneurin on the concentration of aneurin in and the total aneurin content of rat liver. Female rats, 33–35 days old at commencement of experiment, divided into three groups of three rats each, fed diet B plus 6.9, 10, 20 $\mu\text{g.}$ aneurin per day, respectively. Diet B provided additional 0.7 $\mu\text{g.}$ aneurin per day. Rats killed after 28 days, when still increasing in weight. Each point represents a single rat: $\bullet$, $\mu\text{g.}$ aneurin/g. liver; $\circ$, $\mu\text{g.}$ aneurin in whole liver. Mean body weights: 43.8 g. at commencement of experiment, 77.6 g. at end.

Effect of sulphadiazine and succinylsulphathiazole on rats fed diet A. It was essential to investigate the possibility that the effect of sulphadiazine on the aneurin metabolism of rats fed diet A was an indirect consequence of the folic acid and biotin deficiency which inevitably occurs when sulphonamides are included in such a purified diet. Consequently, the

effect of sulphadiazine, which is readily absorbed from the intestine, was compared with that of succinylsulphathiazole, which is only slightly absorbed. The two drugs would be expected to have essentially the same effect on the intestinal bacteria (the non-absorbable drug might possibly cause a greater bacteriostasis than the absorbable), while only the drug which is absorbed could be expected to have a marked direct effect on the tissues of the animal.

The experiment described in Table 2 was carried out to test the effect of these two drugs on both the aneurin and riboflavin economy of the rat. Consequently, the amounts of aneurin (6.9 $\mu\text{g./day}$) and riboflavin (10.3 $\mu\text{g./day}$) given were distinctly less than required for adequate nutrition. Histological examination of the kidneys failed to show any evidence of damage, which is confirmed by the fact that the kidneys of the rats fed sulphadiazine were no heavier than those of the control group. Kornberg, Endicott, Daft & Sebrell (1945) found that kidneys damaged by feeding excessive amounts of the soluble sulphonamides were abnormally heavy.

by sulphadiazine and (2) to determine if the increased aneurin concentration found in the liver was confined to that tissue alone or was also found in other tissues. The rats received the same amount of aneurin as in the first experiment, but the amount of riboflavin was reduced to 6.9 $\mu\text{g./day}$, in order to determine if the failure to find any effect of sulphadiazine on the riboflavin economy in the first experiment was due to the rat receiving so much riboflavin that its tissues were virtually saturated. The concentrations of riboflavin in the liver in the previous experiment were practically the same as those found by Kratzing (1947) in rats fed 30 $\mu\text{g./day}$.

The most important conclusion to be drawn from Table 3 is that PAB, in an amount equimolar with the sulphonamide, has no significant effect, either on its own, or in reversing that of sulphadiazine. Sulphadiazine, itself, had essentially the same effect as in the previous experiment. The only difference is that the effect in the weight of the liver and the kidney is not statistically significant in this experiment. This is, however, not inconsistent with the

Table 2. *Effect of sulphadiazine and succinylsulphathiazole on rats fed diet A*

(Twelve female rats, aged 29 days, were divided into three groups, each containing one rat from each of four litters. Group 1 was fed diet A; group 2, diet A + 0.55% sulphadiazine; group 3, diet A + 0.55% succinylsulphathiazole. Each rat received orally 6.9 $\mu\text{g.}$ aneurin and 10.3 $\mu\text{g.}$ riboflavin/day. The weight of the rats had reached a plateau by the 23rd day of the experiment and remained practically constant for the following 9 days, after which (on the 32nd day) the animals were killed and the livers analysed.)

	Mean of rats in			Statistical significance of difference between any two group means*	Smallest difference between group means which is statistically significant at level	
	Group 1	Group 2	Group 3		$P=0.05$	$P=0.01$
Gain in wt. at end of experiment (g.)	27.2	27.5	27.0	NS	—	—
Liver wt. (% body wt.)	3.84	4.23	3.72	S	0.31	0.46
Kidneys wt. (% body wt.)	1.12	1.00	1.11	S	0.065	0.099
Aneurin content ($\mu\text{g./g.}$ liver)	3.71	5.79	3.54	S	1.01	1.53
Aneurin content ($\mu\text{g./whole}$ liver)	9.8	16.6	8.9	S	2.3	3.6
Riboflavin content ($\mu\text{g./g.}$ liver)	33.1	30.0	32.2	NS	—	—
Riboflavin content ($\mu\text{g./whole}$ liver)	87	86	81	NS	—	—

* S = significant; NS = not significant.

Table 2 shows that sulphadiazine caused the following changes, all of which were statistically significant: (1) the weight of the liver was increased by 10%; (2) the weight of the kidneys was decreased by 11%; (3) the concentration of aneurin in the liver was increased by 56%, and the total aneurin in the liver by 69% compared with group 1; the corresponding percentage increases were 64 and 87% when group 3 was taken as the basis of comparison. On the other hand, succinylsulphathiazole had none of these effects. Neither drug affected the riboflavin content of the liver.

Effect of PAB, both alone and in conjunction with sulphadiazine, on rats fed diet A. The experiment described in Table 3 had two objects in view, (1) to determine if PAB had any effect on the upset caused

findings in the first experiment, but is due to the fact that the variation between individual animals of the same sex in the same litter was much greater in this experiment than in the first, when more animals were available so that more careful matching of the animals with respect to initial body weight was possible. Subsequent experiments (see p. 29) confirmed the effect of sulphadiazine on the weight of the liver and kidney found in the previous experiment. The analyses of the rat minus the liver show that the effect of sulphadiazine on the tissues is not confined to the liver. The concentration of aneurin in the whole rat less its liver was 33% greater in rats fed the drug, compared with that of control rats (combined groups 3 and 4 versus combined groups 1 and 2), and this difference was statistically significant.

Table 3. *Effect of p-aminobenzoic acid (PAB), alone or in conjunction with sulphadiazine, on rats fed diet A*

(Fifteen rats, aged 26–31 days, were arranged in four groups, three groups (no. 1, 2 and 4) containing one rat from each of four litters, while the other group (no. 3) contained one rat from each of three of these litters. The rats from three of the litters were all male, those from the other litter were all female. Group 1 received diet A; group 2, diet A + 0.3% PAB (equimolar with 0.55% sulphadiazine); group 3, diet A + 0.55% sulphadiazine; group 4, diet A + 0.55% sulphadiazine + 0.3% PAB. Each rat received orally 6.9 µg. aneurin and 6.9 µg. riboflavin/day. Maximum weights were reached between the 15th and 18th day; weights then declined rather rapidly for a few days, but were practically stationary for 2–3 days before the termination of the experiment (23rd and 24th days).)

	Mean of rats in				Statistical significance of difference between any two group means†	Smallest difference between group means which is statistically significant at level	
	Group 1	Group 2	Group 3*	Group 4		P = 0.05	P = 0.01
Gain in wt. at end of experiment (g.)	17.5	17.9	18.0	17.6	NS	—	—
Liver wt. (% body wt.)	4.15	4.10	4.08	4.30	NS	—	—
Kidneys wt. (% body wt.)	1.23	1.18	1.08	1.12	NS	—	—
Aneurin content (µg./g. liver)	3.21	3.47	5.94	5.24	S	0.81	1.27
Aneurin content (µg./whole liver)	9.2	9.3	16.1	15.5	S	2.0	3.1
Aneurin content (µg./g. whole rat less liver)	0.81	0.82	1.07	1.10	S	0.23	0.37
Aneurin content (µg./whole rat less liver)	47	47	62	64	S	10	16
Riboflavin content (µg./g. liver)	24.9	25.4	27.7	26.2	NS	—	—
Riboflavin content (µg./whole liver)	73	69	77	78	NS	—	—
Riboflavin content (µg./g. whole rat less liver)	3.19	3.14	2.76	2.74	S	0.40	0.61
Riboflavin content (µg./whole rat less liver)	189	181	164	160	S	20	31

* Mean after estimating 'missing' value by technique of Goulden (1939).

† S = significant; NS = not significant.

As in the previous experiment, the drug had no appreciable effect on the riboflavin in the liver, although the values were considerably lower than in the first experiment, so that any effect of sulphadiazine on the riboflavin values similar to that on the aneurin should have been revealed in this experiment. Sulphadiazine had a small but statistically significant effect on the riboflavin content of the rat carcass from which the liver was removed. This effect, which amounted to only 12%, was in the opposite direction to that on the aneurin content. It may be due to suppression of bacterial synthesis of riboflavin by the drug, although one might expect that PAB would reverse such a suppression and this was not found. It is interesting to note that the riboflavin contents of both the liver and of the whole rat are very much higher than the corresponding values for aneurin, despite the fact that it is generally considered that the riboflavin requirement of animals is about 50% more than that for aneurin. This is an illustration of the fact already discussed by Ferrebee & Weissman (1943) and Kratzing (1947) that tissue catabolism, caused in this case by the deficiency of aneurin, has a strong sparing action on the rat's requirement for riboflavin.

Effect of sulphadiazine on rats fed diet B. Since it now seemed likely that sulphadiazine had little effect on the riboflavin economy of the rat, an optimal concentration of this vitamin was included in the basal diet, which also included the liver powder to provide

adequate amounts of biotin and folic acid. Thus the basal diet was adequate in all the known dietary essentials with the exception of aneurin, 7.9 µg. of which was provided orally per day. The experiment reported in Table 4 showed that the effect of sulphadiazine was the same as in the earlier experiments when the diet was deficient in folic acid and biotin. This result was confirmed in a second experiment in which two groups, each of six young male rats, were fed diet B, giving a total aneurin intake of 8.3 µg./day, and the experimental group received 0.33% sulphadiazine. After 21 days the control animals contained 4.11 µg./g. aneurin in the liver (14.4 µg. aneurin in the whole liver) and those of the experimental group contained 5.16 µg./g. aneurin in the liver (20.6 µg. aneurin in the whole liver). The increase in the aneurin content per gram liver and total aneurin content of liver were statistically significant ($P < 0.001$).

The effect of sulphadiazine on rats receiving much more aneurin (20.7 µg./day) was also studied. The control animals contained 6.84 µg./g. liver (27.9 µg. in the whole liver), while those receiving the drug contained 8.71 µg./g. (37.6 µg. total aneurin). Thus the absolute increase was of the same order as in the experiments with a smaller amount of aneurin.

Comparison of effects of oral and injected sulphadiazine on the aneurin content of the liver and the pyruvate content of the blood, using diet B. Since the metabolism of pyruvate is impaired in aneurin

Table 4. *Effect of sulphadiazine on rats fed diet B*

(Fourteen female rats, aged 23–28 days, divided into two groups, each containing one rat from each of seven litters. Group 1 was fed diet *B*, group 2, diet *B* + 0.55% sulphadiazine. Each rat received orally a supplement of 6.9 µg. aneurin/day. The diet provided 1.0 µg. aneurin/day, so that total intake was 7.9 µg. The rats were killed on the 28th day of the experiment.)

	Mean of rats in		Smallest difference between group means which is statistically significant at level	
	Group 1	Group 2	$P=0.05$	$P=0.01$
Gain in wt. at end of experiment (g.)	40.8	40.1	1.0	1.5
Liver wt. (% body wt.)	3.68	4.26	0.62	0.93
Kidneys wt. (% body wt.)	1.07	0.96	0.05	0.08
Aneurin content (µg./g. liver)	4.89	6.05	0.71	1.08
Aneurin content (µg./whole liver)	11.9	16.8	2.4	3.6
Aneurin content (µg./g. rat less liver)	0.35	0.58	0.17	0.26
Aneurin content (µg./whole rat less liver)	22	36	10	14

Table 5. *Comparison of effects of oral and injected sulphadiazine on the aneurin content of the liver and the pyruvate content of the blood, using diet B*

(Exp. 1. Eighteen male rats, 23–31 days old, divided into three groups of six rats each. Group 1 was fed diet *B*; group 2, diet *B* + 0.55% sulphadiazine; group 3, diet *B*. Group 3 rats were injected four times daily with 12.5 mg. of sulphadiazine (as Na salt), i.e. total of 50 mg./day. All rats received a total of 7.6 µg. aneurin/day. Experiment terminated on 28th day. Blood samples for determination of sulphadiazine and pyruvate taken at time of killing.)

	Mean of rats in			Statistical significance of difference between any two group means*	Smallest difference between group means which is statistically significant at level	
	Group 1	Group 2	Group 3		$P=0.05$	$P=0.01$
Average daily intake of sulphadiazine (mg.)	0	32.2	50	—	—	—
Gain in wt. at end of experiment (g.)	30.5	28.5	28.7	NS	—	—
Sulphadiazine (mg./100 ml. blood)	—	6.2	8.9	S	2.4	3.8
Liver wt. (% body wt.)	4.12	4.31	4.44	NS	—	—
Kidneys wt. (% body wt.)	1.079	0.986	1.055†	S	0.041	0.059
Aneurin content (µg./g. liver)	3.89	6.79	6.66	S	1.01	1.43
Aneurin content (µg./whole liver)	12.1	21.3	21.7	S	2.49	3.53
Pyruvate (mg./100 ml. blood)	2.40	1.30	0.84	S	0.40	0.56

* S = significant; NS = not significant.

† Omitting one rat in this group which had abnormally heavy kidneys and filling 'missing value' by method of Goulden (1939).

(Exp. 2. Seventeen male rats, aged 35–39 days, divided into three groups, groups 1 and 2 of six rats each; group 3, five rats. Group 1 fed diet *B*; group 2, diet *B* + 0.55% sulphadiazine; group 3, diet *B*. Group 3 rats were injected with 15 mg. sulphadiazine (as Na salt) once a day. All rats received a total of 7.85 µg. aneurin/day. Experiment terminated on the 21st day. Blood samples for determination of sulphadiazine and pyruvate taken at time of killing.)

	Mean of rats in			Statistical significance of difference between group means†	Smallest difference between group means which is statistically significant at level	
	Group 1	Group 2	Group 3*		$P=0.05$	$P=0.01$
Av. daily intake of sulphadiazine (mg.)	0	44.2	15	—	—	—
Gain in wt. at end of experiment (g.)	27.7	25.4	27.7	S	1.2	1.7
Sulphadiazine (mg./100 ml. blood)	—	11.5	4.3	S	5.0	7.4
Liver wt. (% body wt.)	4.31	5.05	4.51	S	0.25	0.37
Kidneys wt. (% body wt.)	1.007	0.961	1.092	S	0.068	0.112
Aneurin content (µg./g. liver)	3.68	5.19	4.35	S	1.02	1.46
Aneurin content (µg./whole liver)	13.3	21.0	16.3	S	3.6	5.2
Pyruvate (mg./100 ml. blood)	3.96	2.96	3.20	S‡	0.79‡	1.05‡

* Mean after estimating 'missing' value by technique of Goulden (1939).

† S = significant; NS = not significant.

‡ The standard error used in the calculation of the significance was, in this case, derived from pooling all the blood pyruvate determinations in this and other experiments.

deficiency, pyruvate accumulates in the blood and tissues of aneurin-deficient animals (Thompson & Johnson, 1935). It was therefore of interest to determine whether the increased tissue levels of aneurin resulting from dosage with sulphadiazine were accompanied by a reduced level of pyruvate in the blood. This was one of the objects of the experiments which are summarized in Table 5. The other object was to determine if sulphadiazine had to pass through the alimentary canal before it took effect. The effects of sulphadiazine, given orally and by subcutaneous injection, were compared. In Exp. 1, a total of 50 mg. sulphadiazine per day was injected in four separate portions. This procedure was successful in maintaining a high concentration of the drug in the blood; in fact, the concentration of the drug in the blood was 45% greater in the injected animals than in those receiving the drug orally. In Exp. 2, however, 15 mg. of the drug was administered only once per day, and this was insufficient to maintain an appreciable concentration of the drug. In Exp. 1, the rats were fasted overnight before killing in order to obtain more reliable pyruvate values (Lu, 1939). This probably caused a decrease in the amount of sulphadiazine in the blood.

between high blood sulphonamide, high liver aneurin and low blood pyruvate is clearly shown in Table 6, which is calculated from the figures in Table 5. Owing to different experimental conditions, the figures obtained in the different experiments are not directly comparable.

As in previous experiments, the livers of animals given sulphonamide were heavier than those of the controls and this difference is also independent of the method of administration of the drug. The small amount of drug given to group 3 in Exp. 2 produced a small increase in the liver weight, which was nearly statistically significant at the $P=0.05$ level. The kidneys of the injected animals were definitely heavier than those of the rats fed the drug and also than those of the control rats in Exp. 2. This was not found in Exp. 1, except in one rat of group 3, in which the kidneys were about twice as heavy (expressed as percentage of body weight) as those of the other rats. This animal grew much more slowly than the others after the first 2 weeks of the experiment, so that during the last 2 weeks, it was receiving considerably more sulphonamide per unit body weight than the other rats in this group. Its blood sulphadiazine was the highest in the experiment.

Table 6. *Effect of sulphadiazine. Summary of figures in Table 5*

Exp. no.	Method of administration of drug	Change from control group of		
		Blood sulphadiazine (mg./100 ml.)	Blood pyruvate (mg./100 ml.)	Liver aneurin (μ g./g.)
1	Oral	+6.2	-1.1	+2.9
	Injected (4 $\times$ 12.5 mg./day)	+8.9	-1.6	+2.8
2	Oral	+11.5	-1.0	+1.5
	Injected (1 $\times$ 15 mg./day)	+4.3	-0.8	+0.7

The following conclusions may be drawn from Table 5: (1) High concentrations of sulphadiazine in the blood are accompanied by increased tissue levels of aneurin and reduced blood pyruvate. There was a highly significant negative correlation ($r = -0.76$; $P < 0.001$) between the blood-pyruvate level and the aneurin content of the whole liver in all rats in Exp. 1. (2) The sulphadiazine had essentially the same effect whether it was fed or injected. In Exp. 1 where more sulphadiazine was injected than fed, with the result that the blood sulphadiazine was higher in the injected animals than in those given the drug orally, the blood pyruvate was significantly lower in the former than in the latter. Both groups had approximately the same amount of aneurin in the livers. In Exp. 2, in which considerably less sulphadiazine was injected than fed, which is reflected in the blood sulphadiazine values, the aneurin content of the liver and the pyruvate in the blood of the injected animals were intermediate between the control animals and those fed the drug. The correlation

The greater kidney weight is very probably due to kidney damage caused by precipitation of the drug in the kidney tubules (Kornberg *et al.* 1945). The liver aneurin content of this rat was little different from that of other rats given sulphadiazine, which indicates that the toxic action of sulphadiazine caused by kidney damage is not connected with the effect on the tissue aneurin levels. This rat, which was receiving the most sulphadiazine, also had the heaviest liver (5.0% body weight compared with 4.12% in the control group).

Effect of different sulphonamides and 2-amino-4-methylpyrimidine. In Table 7, the effects of sulphadiazine are compared with those of equimolecular amounts of other sulphonamides, viz. sulphanilamide, sulphapyridine, sulphathiazole and sulphamerazine. The following conclusions may be drawn: (1) Sulphamerazine, like sulphadiazine, caused a considerable increase in the aneurin content of the liver and a decrease in the blood pyruvate and kidney weight. These effects of sulphamerazine

Table 7. *Effect of different sulphonamides*

(Twenty-four male rats, 23–29 days old, divided into six groups of four each, fed diet *B* for 28 days. Total aneurin intake 7.6 μ g./day. Group 1, control diet; group 2, diet + 0.38 % sulphanilamide; group 3, diet + 0.55 % sulphapyridine; group 4, diet + 0.55 % sulphadiazine; group 5, diet + 0.56 % sulphathiazole; group 6, diet + 0.58 % sulphamerazine. 'Paired feeding' method used. Blood samples for pyruvate analyses taken at time of killing.)

	Mean of rats in groups						Statistical significance of difference between group means*	Smallest difference between group means which is statistically significant at level	
	1	2	3	4	5	6		$P=0.05$	$P=0.01$
Gain in wt. at end of experiment (g.)	31.8	34	29.8	36.0	36.8	31.2	S	4.7	6.5
Liver wt. (% body wt.)	4.16	4.01	4.59	4.11	4.37	4.50	S	0.36	0.50
Kidneys wt. (% body wt.)	1.07	1.09	1.13	0.94	1.03	0.85	S	0.10	0.14
Thyroid wt. (mg.)	10.4	10.0	13.3	29.1	10.0	30.8	S	5.6	7.7
Aneurin content (μ g./g. liver)	3.52	3.92	3.74	5.44	3.70	6.25	S	0.72	1.00
Aneurin content (μ g./whole liver)	10.9	12.3	12.8	17.9	13.2	21.4	S	2.4	3.4
Pyruvate (mg./100 ml. blood)	2.5	2.1	2.5	1.8	2.6	1.4	S	1.0†	1.3†

* S = significant; NS = not significant.

† The standard error used in the calculation of the significance was, in this case, derived from pooling all the blood-pyruvate determinations in this and in other experiments.

Table 8. *Effect of 2-amino-4-methylpyrimidine on rats*

(Eight male rats, 39 days old, divided into two groups of four each, fed diet *B* for 21 days. Total aneurin intake, 7.8 μ g./day. Group 1, control diet; group 2, diet + 0.24 % 2-amino-4-methylpyrimidine. 'Paired feeding' technique.)

	Mean of rats in		Smallest difference between group means which is statistically significant at level	
	Group 1	Group 2	$P=0.05$	$P=0.01$
Gain in wt. at end of experiment (g.)	33.6	37.8	3.5	6.5
Liver wt. (% body wt.)	5.75	5.95	0.46	0.84
Kidneys wt. (% body wt.)	1.05	0.98	0.05	0.09
Thyroid (mg.)	5.65	7.3	1.9	3.4
Aneurin content (μ g./g. liver)	3.33	3.07	0.74	1.37
Aneurin content (μ g./whole liver)	15.9	14.8	5.7	10.4
Pyruvate (mg./100 ml. blood)	1.9	1.8	0.4	0.7

were, in fact, somewhat greater than those of sulphadiazine. (2) Sulphanilamide had no effect, sulphapyridine caused a significant increase in the liver weight, but otherwise had no effect, while the only significant effect of sulphathiazole was on the weight gain (all groups received the same food intake).

In this experiment the thyroids were weighed, since Mackenzie & Mackenzie (1943) had shown that certain sulphonamides caused an increased weight of the thyroid. It is most noticeable that the two drugs which had the effect on the aneurin and pyruvate levels were the only two to increase the thyroid weight and this increase amounted to trebling the weight.

Since the only drugs which had the effect studied in this paper were sulphadiazine and the very closely related sulphamerazine (which differs from sulphadiazine only in having one methyl group substituted in the pyrimidine ring), the effect of the same concentration of the simple pyrimidine, 2-amino-4-methylpyrimidine, was tried in the experiment

summarized in Table 8. This compound was, however, without any effect on the aneurin and pyruvate levels. It appeared to have some stimulatory effect on growth and caused a lower kidney weight (expressed as a percentage body weight). The comparison of the kidney weights in this experiment is, however, complicated by the difference in the weight of the animals.

Survival experiments. Two groups of ten male rats (30–36 days old) were fed *ad lib.* a diet the same as diet *B* except that it provided 3 μ g. aneurin/10 g. dry weight of food; no additional aneurin was provided. One group received 0.33 % sulphadiazine in the diet. Both groups survived for about the same time, approximately 40 days, but it was noticeable that the typical aneurin-deficiency symptoms were shown only by the rats in the control group. These symptoms were: (1) drop in food consumption, (2) cessation of growth followed by fairly rapid decline in weight, (3) typical hump back, (4) rough fur, (5) the onset of priapism in some, and (6) lack of co-

ordination and convulsions. The rats fed the drug, on the other hand, lost much less weight (only about 10 % of the maximum) and did not show these typical signs of aneurin deficiency. They remained in a crouched position for long periods of time, all movements apparently being painful; the fur was in good condition, but felt cold. The animals lived 3-5 days after the onset of these toxic symptoms and died of convulsive seizures.

DISCUSSION

From the above experiments it can be concluded that the inclusion in the diet of 0.55 % sulphadiazine or sulphamerazine (but not of equimolar amounts of the other sulphonamides tried) has a marked effect on the aneurin economy of young rats. This is characterized, not only by an increased concentration of aneurin in the tissues of the animals fed the sulphonamide, but also by decreased blood-pyruvate levels. Rats on a very low aneurin diet, when fed sulphadiazine, did not show the typical aneurin-deficiency symptoms seen in controls not given the drug. The magnitude of the effect on the aneurin economy in a typical experiment (Exp. 1, Table 5) can be calculated from the increase in the aneurin content of the liver in this experiment and the relation between aneurin intake and liver content shown in Fig. 1. This calculation showed that supplementing the diet with 0.55 % sulphadiazine was equivalent to increasing the aneurin intake from 7.6 to 15.0 $\mu\text{g./day}$, i.e. to very nearly doubling the aneurin intake.

No systematic study was made of the effect of different amounts of drug or of the time of treatment on the effect on the aneurin economy, but there are indications that 0.55 % sulphadiazine in the food is probably nearly sufficient for the maximum effect. Thus, when the amount of sulphonamide given was increased (Exp. 1, Table 5), there was no appreciable increase in the aneurin content of the liver (although the blood-pyruvate level was somewhat lower). On the other hand, when less sulphadiazine was injected (Exp. 2, Table 5) the effect on the aneurin and pyruvate levels was appreciably less.

During the course of this investigation, several possible explanations of this effect were considered, but were eliminated as the result of further experiments. These were: (1) an effect on the renal threshold of aneurin, (2) change of bacterial flora in the intestine with altered vitamin synthesis, (3) a consequence of the extensive acetylation of the sulphonamide, and (4) an inhibition of intracellular enzymes containing aneurin.

(1) *Renal threshold.* If the sulphonamide increased the renal threshold of aneurin, increased aneurin concentrations in the tissues might be expected. However, an increased renal threshold would cause a decrease of the aneurin excretion

which is contrary to the finding previously reported (Slater, 1946) that, in man, the urinary excretion of aneurin was greatly raised by ingestion of sulphadiazine. Kinnunen (1946) found that a number of sulphonamides did not affect the aneurin excretion of rats, but this is not inconsistent with the present findings, since Kinnunen did not use either sulphadiazine or sulphamerazine, which were the only two sulphonamides found effective in the present study. In any case, Kinnunen used aneurin-deficient rats in these experiments and a considerable change in the aneurin economy of such rats would not be reflected by an appreciable rise in the urinary excretion of aneurin.

(2) *Bacterial synthesis.* Ellinger and his co-workers (Ellinger & Emmanuelowa, 1946; Ellinger, Abdel Kader & Emmanuelowa, 1947) have shown that the administration of ambamide (*p*-aminomethylbenzenesulphonamide) increased the urinary excretion of *N*-methylnicotinamide by man and rats. This is due to a change of the intestinal flora, brought about by the drug, leading to an increased intestinal synthesis of nicotinamide. It was possible that sulphadiazine had a similar effect on the bacterial synthesis of aneurin. This was, however, considered unlikely for the following reasons: (a) The therapeutic sulphonamides suppress rather than increase the intestinal synthesis of the B vitamins. (b) The amount of aneurin available to the rat from intestinal synthesis when fed a high sucrose diet of the type used in these experiments must be very small, since rats very soon die of aneurin deficiency, unless the diet is supplemented with aneurin. (c) Since succinylsulphathiazole is not absorbed, but remains in the intestine, and has much the same type of effect on bacteria as sulphadiazine, it would be expected, on this hypothesis, that succinylsulphathiazole would have a greater effect on the aneurin levels than sulphadiazine. Actually it was found (Table 2) that succinylsulphathiazole had no effect. (d) Injected sulphadiazine, which would have little action on the intestinal bacteria, was just as effective as that orally administered in raising the aneurin levels. This experiment also excluded the possibility that the effect of sulphadiazine was on the absorption of the orally administered aneurin.

(3) *Acetylation.* Sulphonamides are extensively acetylated *in vivo*, particularly in the liver. Klein & Harris (1938), James (1939, 1940), Martin & Rennebaum (1943) and Kinnunen (1946) have shown that this acetylation is dependent upon the supply of pyruvate or other intermediary metabolites, which, according to widely accepted views, are converted into a C_2 compound *in vivo*. According to Kinnunen (1946), it is this C_2 compound which is responsible for the acetylation, and sulphonamides compete with oxalacetate for the C_2 compound. Thus malate, which is converted to oxalacetate, reduces the

acetylation of sulphonamides, while the latter inhibit the formation of citrate from oxalacetate. Martin & Rennebaum (1943), Lipmann & Kaplan (1946) and Kinnunen (1946) have all implicated aneurin in the acetylation, since aneurin-deficient animals acetylate sulphonamides poorly, and this is increased both *in vivo* and *in vitro* by the injection of aneurin or by the addition of cocarboxylase to liver preparations (cf. also the effect of aneurin on acetylcholine synthesis; Mann & Quastel, 1939). Kinnunen (1946) considers that the effect of aneurin deficiency is due to a reduction in the amount of C_2 compound formed.

It is necessary, then, to consider the possibility that the effect of sulphonamides on the aneurin economy described in the present paper is a consequence of their acetylation. According to Kinnunen (1946), the reaction limiting both the acetylation of sulphonamides and the condensation of the C_2 compound with oxalacetate would be, in the aneurin-deficient animal, the conversion of pyruvate into the C_2 compound. It is difficult to see why the introduction of the sulphonamide, which competes with the oxalacetate for the C_2 compound, should affect the conversion of pyruvate into the C_2 compound. It would only reduce the rate of the Krebs cycle and this to only a very slight extent, since 100 times as much carbohydrate as sulphonamide was fed to the animal. Thus the acetylation of sulphonamide provides no explanation for the lower blood-pyruvate and higher tissue-aneurin levels found in the present investigation. Moreover, PAB, which is acetylated in the same way as sulphonamides (Doisy & Westerfeld, 1943), had no effect whatever on the tissue-aneurin levels (Table 3). Table 7 shows also that the effect on aneurin economy is not common to all sulphonamides, although these are all acetylated in essentially the same manner (Kinnunen, 1946).

(4) *Inhibition of aneurin-containing enzymes.* Sulphadiazine and sulphamerazine, which were the only two sulphonamides affecting aneurin economy, are chemically related to aneurin. It seemed possible then that these drugs displaced aneurin from some enzyme containing aneurin or a derivative as prosthetic group. There is, in fact, some evidence that sulphonamides can act in this way. Sevag, Shelburne & Mudd (1942, 1945) have shown that certain sulphonamides inhibit yeast and bacterial carboxylases and that this inhibition is reversed by cocarboxylase. However, there are a number of reasons which indicate that such an inhibition of an aneurin-containing enzyme cannot be the explanation of the present results: (1) Sevag *et al.* (1942) found that sulphathiazole was much more inhibitory on carboxylase than other sulphonamides, whereas sulphathiazole had no effect in the present experiments; (2) Sevag *et al.* (1942) required very much higher concentrations of sulphonamide than would be possible in mamma-

lian tissues; (3) although such a displacement of aneurin from an enzyme might lead to raised tissue levels of aneurin, it is difficult to see why probably the most important *in vivo* function of an aneurin-containing enzyme, viz. the metabolism of pyruvate, should apparently be enhanced. Frohman & Day (1949) have found that injection of hydroxyaneurin (oxythiamine), an aneurin antagonist, into rats caused an increase both of the urinary excretion of aneurin and of the blood pyruvate.

None of the above explanations satisfactorily dealt with the finding that the increased tissue levels of aneurin were accompanied by lower blood-pyruvate levels and in the human experiment by an increased urinary excretion. Sulphadiazine acts, in all these three respects, by nullifying the effects of the aneurin deficiency, i.e. sulphadiazine has a true aneurin-sparing action on the animal. In general, there are three ways by which the drug could have this effect: (1) by replacing aneurin in some enzyme system; (2) by preventing the destruction of aneurin; (3) by altering the metabolism in such a way as to decrease the animal's requirement of aneurin. The first explanation is difficult to reconcile with the known specificity of aneurin in biological systems. While it is not possible to exclude the second explanation, it is rather unlikely since it implies that at least half of the aneurin provided to animals not fed the drug is destroyed. The third explanation is believed to be the most likely and will be considered in greater detail. Since the drug is effective in relatively low concentrations, its effect on metabolism is most likely due to an interference with some catalytic mechanism, rather than to direct intervention as a metabolite in the metabolic processes, e.g. by competing with oxalacetate for the C_2 compound. The delay in reaching the maximum excretion of aneurin with the human subject given sulphadiazine (Slater, 1946) rather suggests that the action of the drug might be indirect. Although sulphanilamide has some depressing effect on the respiration of spermatozoa (Knott & Salisbury, 1946), the sulphonamides which have been tried either *in vivo* or *in vitro* do not appear to have any marked effect on the respiratory enzymes in tissue preparations (Rerabek, 1943; Anderson, Pilgrim & Elvehjem, 1944), except glucose-6-phosphate dehydrogenase, which is inhibited by a number of sulphonamides (Altman, 1946). However, sulphadiazine or sulphamerazine were not used in the studies of Rerabek or of Anderson *et al.* (1944). Altman (1946) found that sulphadiazine was considerably less inhibitory than sulphanilamide, sulphapyridine or sulphathiazole. Thus, none of these studies provides any clue as to the mechanism of the effects described in the present paper. Similarly, these cannot be connected with the inhibition of carbonic anhydrase, since sulphonamides substi-

tuted in the sulphonamide group do not inhibit this enzyme (Mann & Keilin, 1940).

Mackenzie & Mackenzie (1943) and Astwood, Sullivan, Bissell & Tyslowitz (1943) have shown that certain sulphonamides, when fed to rats, produced a remarkable enlargement of the thyroid gland, which was prevented by thyroxine. The thyroid hypertrophy and hyperplasia were accompanied by a marked drop in the basal metabolic rate (20 % after 14 days). Sulphadiazine was by far the most effective sulphonamide. Franklin & Chaikoff (1944) have found that sulphonamides interfere directly with the conversion of inorganic iodide to diiodotyrosine and thyroxine by surviving slices of thyroid tissue.

This effect of sulphadiazine on the weight of the thyroid was confirmed by the experiment described in Table 7. Sulphamerazine had the same effect. In the doses employed, sulphapyridine had a suggestive but statistically non-significant effect while sulphanilamide and sulphathiazole were ineffective. It was striking that the only two drugs which had this marked effect on the thyroid were also the only two which caused raised tissue-aneurin and lowered blood-pyruvate levels. This suggests the possibility that the effect of sulphadiazine and sulphamerazine on the aneurin economy is a consequence of the lowered basal metabolic rate caused by the effect on the thyroid. This hypothesis should be tested by trying the effect on the aneurin and pyruvate levels of other substances, such as thiourea, which also decrease the synthesis of thyroxine.

The incorporation of both liver powder and PAB (0.01 %) into the diet did not prevent the thyroid enlargement (this was also found by Mackenzie & Mackenzie, 1943) or the increased tissue aneurin produced by sulphadiazine. A much larger percentage of PAB (0.3 %, equimolar with the sulphadiazine) was also found to have no effect on the amount of aneurin in the tissues. This lack of effect of such a large dose of PAB as well as the fact that not all sulphonamides have the two effects is a clear indication that the reaction with which sulphadiazine is concerned in animal tissues is very different from that responsible for the bacteriostatic action of sulphonamides. In fact, none of the effects of sulphonamides on animals appear to be affected by PAB, with the exception of those toxic symptoms which are caused by the suppression by the sulphonamide of the bacterial synthesis of a vitamin in the intestine (Black, Overman, Elvehjem & Link, 1942; Kornberg, Daft & Sebrell, 1944; Petering, Delor & Murray, 1947).

It is interesting that sulphadiazine did not have any effect on the riboflavin economy of the rat. This suggests that the supply of riboflavin is much less critical than that of aneurin for the metabolism of pyruvic acid.

It is noteworthy that those drugs which had the

effect on the thyroid and on the aneurin economy also caused a small but significantly lower kidney weight (Table 7). This may be a reflection of the improved aneurin nutrition of these animals, since Zucker & Zucker (1946) found that the kidneys of vitamin B-complex deficient rats were 50 % overweight. Usually, the liver of rats fed sulphadiazine was about 10 % heavier than the control rats. The reason for this is not known, but it may be connected with the acetylation which occurs in the liver (cf. Kinnunen, 1946).

The present experiments give no support to the suggestions of several authors (reviewed by Kinnunen, 1946) that the administration of sulphonamides increases the aneurin requirement (except when intestinal synthesis of aneurin provides a significant proportion of the total intake; Najjar & Holt, 1943). In fact, the results show that most sulphonamides have no effect on the aneurin economy, whilst those which do have an effect, viz. sulphadiazine and sulphamerazine, decrease rather than increase the requirement.

SUMMARY

1. A method of determining aneurin in rat liver and rat carcasses is described.
2. An approximately linear relationship was found between the aneurin content of the liver and the dietary intake of aneurin over the range 7.6–20.7 $\mu\text{g./day}$.
3. The inclusion of 0.55 % sulphadiazine in a diet moderately low in aneurin brought about the following changes in young rats compared with controls fed the same diet without the drug: (1) the aneurin content of the liver was about 60 % greater; (2) the aneurin content of the whole rat less liver was also greater; (3) the blood pyruvate was lower by about 30 %; (4) the kidney weight was 10 % less; (5) the liver weight was 10 % greater; (6) the thyroid was three times as heavy.
4. Injections of the drug had the same effect as oral administration.
5. Sulphamerazine behaved in the same way as sulphadiazine, but sulphanilamide, sulphapyridine, sulphathiazole, succinylsulphathiazole and 2-amino-4-methylpyrimidine, in equimolar amounts, did not show this effect.
6. *p*-Aminobenzoic acid (in an equimolar amount) did not behave like sulphadiazine, nor did it prevent the effects of the sulphonamide when the two were fed together. The effect of sulphadiazine was also not influenced by the inclusion or absence of folic acid and biotin in the diet.
7. Sulphadiazine had no appreciable effect on the riboflavin content of the tissues.
8. Inclusion of sulphadiazine in a diet low in aneurin prevented the development of typical

aneurin-deficiency symptoms, although it did not prolong the life of the rats.

9. It is concluded that sulphadiazine has a true aneurin-sparing action on the animal. This cannot be explained on the basis of (1) an effect on the renal threshold of aneurin; (2) change of bacterial flora in the intestine with altered vitamin synthesis; (3) acetylation of sulphonamide; (4) inhibition of intracellular enzymes containing aneurin.

10. It is thought probable that the sulphadiazine, by interfering with some catalytic mechanism,

alters the metabolism in such a way as to decrease the animal's requirement of aneurin.

11. It is suggested that this alteration results from the inhibition of the synthesis of thyroxin caused by certain sulphonamides.

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