ANTIMICROBIAL AGENTS IN THE PREVENTION OF DIETARY HEPATIC INJURY (NECROSIS, CIRRHOSIS) IN RATS*

By PAUL GYÖRGY, M.D., JOSEPH STOKES, Jr., M.D., HARRY GOLDBLATT, M.D., and HANS POPPER, M.D.

(From the Nutritional Service, Department of Pediatrics, School of Medicine, University of Pennsylvania, Philadelphia; Institute for Medical Research, Cedars of Lebanon Hospital, and Department of Pathology, University of Southern California, Los Angeles; and Hektoen Institute for Medical Research, Cook County Hospital, Northwestern University Medical School, Chicago)

(Received for publication, December 15, 1950)

It has been previously shown (1) that aureomycin, when added to a necrogenic yeast diet, has a significant beneficial effect in the prevention of experimental hepatic necrosis in rats. In contrast to vitamin E or the sulfur-containing amino acids, cystine or methionine, which as supplements to the basal experimental yeast diet will permanently prevent the production of hepatic necrosis, the effect of aureomycin was found to be as a rule only temporary and more in way of a delay.

In discussing the possible mechanism of the aureomycin effect it has been emphasized (*a*) that intensive studies have revealed no indication of an underlying infection as the direct cause of so called dietary hepatic necrosis, (*b*) that the choice appeared to lie between a direct metabolic effect of aureomycin on the liver and its antibiotic effect on the intestinal flora, in particular the coliform organisms, and (*c*) that this antibiotic effect in turn may prevent the formation of bacterial metabolites with which the liver, in the absence of vitamin E or of sulfur-containing amino acids, is unable to cope.

The beneficial effect of aureomycin in our experiments was not limited to the delay of hepatic necrosis but manifested itself in a slight but definite stimulation of growth (1).

If the effect of aureomycin is mediated by the suppression of the intestinal flora, other antimicrobial agents should also prove to be effective, although

* Supported in part by the Commission on Liver Disease of the Armed Forces Epidemiological Board, Preventive Medicine Division, Office of the Surgeon General, Washington, D. C., and in part by a research grant (to Dr. Harry Goldblatt) from the Division of Research Grants and Fellowships of the National Institutes of Health, Public Health Service.

A preliminary report on part of this work was given by Dr. Paul György at the 9th Conference on Liver Injury, April, 1950, sponsored by the Josiah Macy, Jr., Foundation, New York.

not necessarily equal to aureomycin, depending mainly on their bacterial "spectrum" and on the ease with which they may produce resistant strains. Thus, it seemed advisable to study the effect of various antimicrobial agents on the production of dietary hepatic necrosis, especially in comparison to that of aureomycin.

In contrast to necrosis, dietary hepatic cirrhosis may be produced in the presence of cystine (not of methionine) or vitamin E (5). In consequence, vitamin E and cystine may not act for this pathologic condition in the same "detoxifying" role as they supposedly do in the prevention of massive hepatic necrosis. It appeared to be of interest to study any possible effect of aureomycin on the development of hepatic cirrhosis in rats fed the usual low protein–high fat diet (5) supplemented with vitamin E.

Experimental Method

In the experiments dealing with the production of massive hepatic necrosis (Tables I to III) the same necrogenic basal diet (Y5H) was used as in our previous studies (1, 6): Yeast (British type of bakers' yeast[1]), 18; corn starch, 79; salt mixture U.S.P. II, 3. Peanut oil, 0.5 ml., and cod liver oil, 2 drops, were added to 8 gm. of the dry mixture just before feeding. Further, all animals received daily supplements of 20 μg. of thiamine, 25 μg. of riboflavin, 20 μg. of pyridoxine, and 100 μg. of calcium pantothenate dissolved together in 1 ml. of water, as well as 20 μg. of vitamin K (menadione). Rats were of the Sprague-Dawley strain, males, with an average initial weight of 50.0 to 56.0 gm. in the various subgroups. In one subgroup of Experiment 50 (Table II), 5 parts of pectin was substituted for equal parts of corn starch, the total percentage of the latter being thus reduced to 74. As antimicrobial supplements[2] we used aureomycin, polymyxin, streptomycin, chloromycetin (synthetic, crystalline), terramycin, penicillin, all in doses of 25 mg. daily per rat. The antibiotics were given by mouth, with the exception of penicillin which was administered by injection. One subgroup in Experiment 51 received sulfaguanidine, admixed to the diet 1:100. The experiments were carried out between November, 1949, and May, 1950.

Streptomyces griseus and *aurefaciens* are producers not only of antibiotics, streptomycin and aureomycin respectively, but also of B_{12}. In consequence, the possibility that aureomycin and streptomycin may contain traces of B_{12} had to be considered. In this connection the fact that yeast, which is the chief constituent of the basal necrogenic diet, is free from B_{12} should also be borne in mind. We tested the effect of B_{12} as supplement (5 μg. daily per rat) in two subgroups of Experiment 50, one with, and the other without, additional aureomycin.

The subgroups in the various experiments consisted of 12 to 14 animals.

In Experiment 53 (Table IV) the effect of aureomycin (25 mg. daily per rat) was tested on the development of dietary hepatic cirrhosis. The ration (LVI) consisted of vitamin-free casein (General Biochemicals, Inc., Chagrin Falls, Ohio), 8; sucrose, 48; lard, 38; cod liver oil, 2; salt mixture (U.S.P. II), 4; niacin, 0.01, with supplements of crystalline B vitamins and of vitamin K as given in the experiments on massive hepatic necrosis. Male and female rats (10 animals in each subgroup) of the Sprague-Dawley strain, weighing approximately 150 gm. were used. All surviving rats were killed after 100 days in the experiment.

[1] "D.C.L. vitamin B_1 yeast" from Distillers Corporation, Ltd., Glasgow C4, Great Britain.

[2] Aureomycin was kindly furnished by the Lederle Laboratories, Inc., polymyxin by Burroughs Wellcome Laboratories, chloromycetin by Parke, Davis and Co., and terramycin by Charles Pfizer and Co.

In all experiments the rats were kept in single cages, with raised, wide-meshed screen bottoms. The daily food intake was measured.

At the end of the experiments an autopsy was performed on all the animals. The findings observable in the gross were recorded; liver, and in Experiment 53, also kidneys, pancreas, and thyroid glands, were subjected to histological examination.

EXPERIMENTAL OBSERVATIONS

Massive Hepatic Necrosis.—The relevant data are summarized in Tables I to III. Compared with our previous observations (1) the survival time of the rats fed the experimental necrogenic diet was considerably reduced, from an average of 141 days (1) to 34 to 43 days in the more recent studies. It may be assumed that this difference is due to the higher average initial weight of the

TABLE I

The Effect of Aureomycin, Streptomycin, and Polymyxin on Massive Hepatic Necrosis. Experiment 48

	Survival time	Average initial weight	Weight gain during first 4 wks.	Food intake (average) during				
				1-4 wks.	5-8 wks.	9-12 wks.	13-16 wks.	17-20 wks.
	days	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Controls (13)	34.3 ± 2.4*	51.0 ± 1.15	4.4 ± 1.3	6.4 ± 0.1	—	—	—	—
Aureomycin (14)	110 ± 13.0	53.2 ± 0.8	24.0 ± 1.05	6.7 ± 0.03	7.0 ± 0.05	6.7 ± 0.03	6.7 ± 0.1	6.5 ± 0.04
Polymyxin (15)	35.9 ± 1.8	54.0 ± 0.9	12.0 ± 1.05	6.5 ± 0.04	—	—	—	—
Streptomycin (14)	50.0 ± 1.15	50.0 ± 0.85	25.5 ± 0.9	6.4 ± 0.03	6.1 ± 0.1	—	—	—

* Standard error of the mean.

experimental animals in the older experiment, 138 gm., contrasted with 50 to 54 gm. in the present experiments. It has been previously shown by Himsworth and Lindan (7) that old rats are more resistant to dietary massive necrosis, presumably because of their high initial vitamin E stocks which will afford a more prolonged protection, in contrast to younger animals with their smaller vitamin E reserves.

Not only the control animals but also the experimental rats receiving supplements of aureomycin to their basal diet showed reduced survival times when compared with that of the animals in the previous study (1). Here again, this finding may be related to the difference in the initial weights. In Experiment 48 (Table I) and Experiment 50 (Table II) the survival rate of the animals receiving supplements of aureomycin was 110 days, that of the control animals 34 to 41 days.

In Experiment 48 (Table I) we compared aureomycin with polymyxin and streptomycin, and found polymyxin ineffective, streptomycin only slightly, and

aureomycin very significantly effective. The delay in the production of massive necrosis by aureomycin becomes particularly evident when the number of surviving animals is charted in relation to days of survival (Fig. 1). As seen

TABLE II

The Effect of Aureomycin, B_{12}, Streptomycin, and Pectin on Massive Hepatic Necrosis. Experiment 50

	Survival time	Average initial weight	Weight gain during first 4 wks.	Food intake (average) during			
				1–4 wks.	5–8 wks.	9–12 wks.	13–16 wks.
	days	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Controls (14)	41.3 ± 1.45	50.0 ± 0.75	7.0 ± 1.1	6.4 ± 0.1	—	—	—
B_{12} (13)	43.4 ± 3.2	50.0 ± 0.8	10.5 ± 1.0	6.6 ± 0.1	—	—	—
Aureomycin (13)	110.7 ± 10.2	50.9 ± 1.15	27.0 ± 1.2	6.6 ± 0.05	6.4 ± 0.05	6.7 ± 0.05	6.8 ± 0.05
Aureomycin and B_{12} (14)	96.8 ± 9.0	50.0 ± 0.7	23.6 ± 0.85	6.8 ± 0.1	6.5 ± 0.05	6.8 ± 0.0	6.8 ± 0.01
Streptomycin (14)	56.0 ± 3.5	50.5 ± 0.8	12.5 ± 0.8	6.6 ± 0.05	6.5 ± 0.1	—	—
Streptomycin and pectin (14)	71.0 ± 6.4	50.0 ± 0.7	14.0 ± 1.1	6.7 ± 0.1	6.5 ± 0.1	—	—

TABLE III

The Effect of Chloromycetin, Terramycin, Penicillin, and Sulfaguanidine on Massive Hepatic Necrosis. Experiment 51

	Survival time	Average initial weight	Weight gain during first 4 weeks.	Food intake (average) during			
				1–4 wks.	5–8 wks.	9–12 wks.	13–16 wks.
	days	*gm.*	*gm.*	*gm.*	*gm.*		
Controls (12)	42.8 ± 1.2	54.0 ± 0.9	6.2 ± 2.0	6.4 ± 0.1			
Chloromycetin (14)	41.3 ± 1.3	54.3 ± 0.65	16.0 ± 1.15	6.5 ± 0.05			
Sulfaguanidine (13)	50.8 ± 3.0	53.0 ± 0.75	14.8 ± 1.5	6.3 ± 0.1	6.1 ± 0.3		
Terramycin (14)	65.0* ± 5.3	53.1 ± 0.55	18.7 ± 1.0	6.2 ± 0.05	6.4 ± 0.1	6.6 ± 0.1	
Penicillin (14)	42.1 ± 2.6	56.0 ± 0.7	17.6 ± 1.15	6.5 ± 0.1	6.8 ± 0.1		

* Four rats included died after 36, 45, 80, and 122 days from volvulus of the sigmoid, without hepatic necrosis.

from the data of Experiment 50 (Table II) supplements of B_{12} had no effect on the production of dietary massive necrosis, regardless of whether it was added to the basal diet (8) or to the basal diet already supplemented with aureomycin. Thus, the effect of aureomycin could not be due to "contamination" with B_{12}. In Experiment 50 the beneficial effect of streptomycin was more pronounced than in Experiment 48, and became even further accentuated in combination with pectin.

In Experiment 51 (Table III) the findings with chloromycetin, sulfaguanidine, terramycin, and penicillin are summarized. Chloromycetin and penicillin were ineffective, whereas sulfaguanidine showed only slight indication of a positive effect. In contrast, terramycin, although not as effective as aureomycin in the previous experiments, was found to exhibit a significant delaying effect on the development of massive hepatic necrosis.

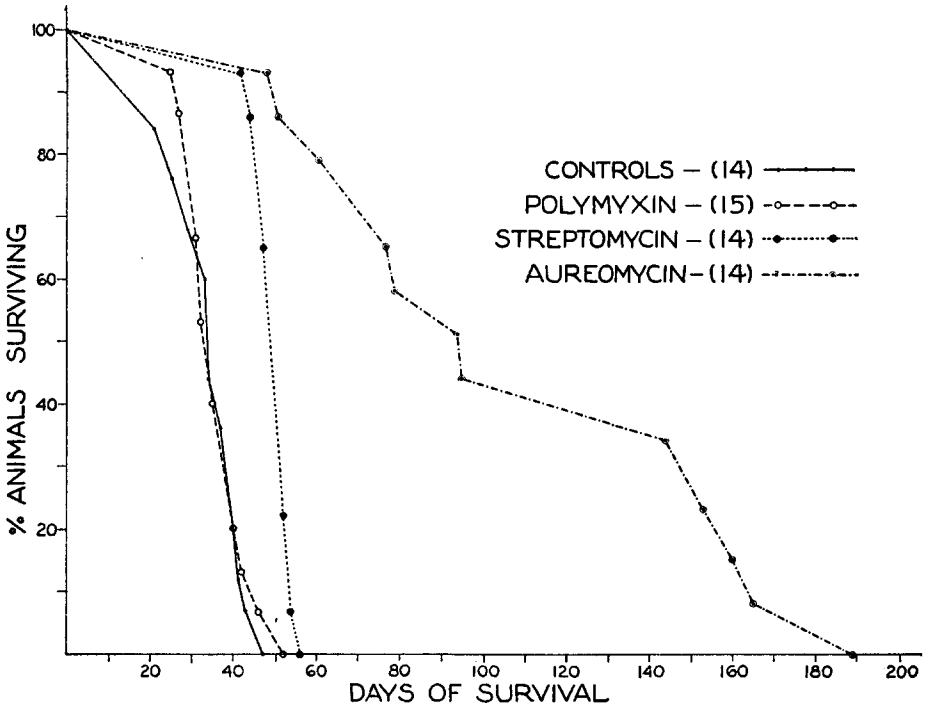

FIG. 1. Days of survival in groups of rats receiving the necrogenic basal diet with and without supplements of antibiotics.

Dilated cecum and colon were often seen in rats receiving supplements of aureomycin or terramycin to the basal yeast diet. The distention of the large intestine was especially pronounced in rats receiving terramycin. Four animals in this group (Experiment 51) appeared to have died from volvulus of the colon (two with perforation), without signs of simultaneous hepatic necrosis. All other rats (control and experimental animals) which died during the course of Experiments 48, 50, and 51 have shown gross and microscopic evidence of massive hemorrhagic necrosis of the liver, with all its characteristic manifestations, including its often demonstrable prevalence in the left half of the liver (6, 9).

All the antimicrobial agents used have stimulated gain in weight of the animals during the first 4 weeks of the experiments (Tables I to III). Although

penicillin, chloromycetin, and polymyxin had no beneficial effect, and sulfaguanidine had barely any beneficial effect on hepatic necrosis, their growth-stimulating effect was as great as that of streptomycin, terramycin, and aureomycin, which—in this order of least to greatest—were found significantly beneficial in delaying the production of massive hepatic necrosis. The difference in growth stimulation could not be correlated with the average daily food intake during the same experimental period (Tables I to III).

Diffuse Hepatic Fibrosis (Cirrhosis).—In two control groups of rats (10 males, 10 females) fed a cirrhosis-producing diet (LVI) cirrhosis was observed in 9 males and in 10 females at the end of the experimental period (100 days), with a high incidence of ascites in both groups (Table IV).

TABLE IV

Incidence of Cirrhosis in Rats Fed a Cirrhosis-Producing Diet with and without Supplement of Aureomycin. Experiment 53

Diet	No. of animals	Sex	Average weight		Food intake (average) during				Cirrhosis*			Ascites	Kidney injury	
			Initial	Final	1-4 wks.	5-8 wks.	9-12 wks.	13-15 wks.	0	±	+		0	+
			gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*						
LVI	10	M	158 ± 2.5	116 ± 11.0	5.7 ± 0.2	4.0 ± 0.2	3.5 ± 0.3	4.3 ± 0.2	1	—	9	5	0	10
LVI and aureomycin	10	M	160 ± 2.9	176 ± 6.5	6.2 ± 0.2	5.3 ± 1.2	5.5 ± 0.2	6.2 ± 0.2	10	—	0	0	10	0
LVI	10	F	157 ± 2.0	123 ± 6.4	6.0 ± 0.2	4.1 ± 0.2	3.7 ± 0.3	3.8 ± 0.4	0	—	10	9	0	10
LVI and aureomycin	10	F	157 ± 1.8	176 ± 3.6	6.5 ± 0.2	5.7 ± 0.2	5.3 ± 0.3	6.1 ± 0.2	7	1	2	1	8	2

* No attempt has been made to grade the cirrhosis beyond the initial (±) and pronounced (+) changes.

The histological examination revealed the typical picture of experimental dietary cirrhosis (5) in accordance with the findings in the gross. Typical manifestations of acute necrotizing nephrosis accompanied the hepatic changes, as previously described (5). No significant alterations were found in the pancreas; the basophilia of the cytoplasm was in all instances well preserved.

Two other groups of rats (10 males, 10 females) received the same basal cirrhosis-producing diet (LVI) as the above group, supplemented with 25 mg. aureomycin (mixed with the diet). As can be readily seen from the tabulated data (Table IV), cirrhotic changes in the liver as well as ascites were conspicuous by their virtual absence. Microscopic examination showed definite but not excessive fat infiltration of liver cells, and, with the exception of 3 rats in the group of female animals, no concomitant cirrhotic changes. In one of the 3 rats with cirrhosis, the fibrotic proliferation was very slight. Renal changes were observed in only two of the animals, with definite cirrhosis of the liver. The pancreas was normal in all the animals.

The average food intake was higher in the groups of animals receiving supplements of aureomycin, especially in the latter part of the experiment. The average weight curve showed decrease in the control animals, and statistically significant increase in the rats receiving supplements of aureomycin.

The thyroid glands were studied microscopically. In both groups, the thyroid glands were not entirely normal. In both, they were the seat of hypertrophy and hyperplasia. This was of very slight degree in the group on the cirrhosis-producing diet alone, and of slight degree in the group that also received aureomycin. The difference between the two groups was, nevertheless, definitely recognizable.

DISCUSSION

In the experiments here reported, our previous findings (1) on the beneficial effect of aureomycin in the partial prevention of dietary massive necrosis of the liver have been confirmed and expanded. It has been shown that whereas young animals fed the basal necrogenic yeast diet succumbed to massive necrosis in about 35 to 42 days, supplements of aureomycin prolonged the survival time up to 110 days.

In analyzing this aureomycin effect, several possibilities might be taken into consideration: (*a*) the presence of a missing, antinecrogenic substance, (*b*) a direct metabolic effect, and, (*c*) an antimicrobial effect on the intestinal flora. As one possible "contaminant" we have used B_{12} as a supplement to the basal diet, with or without added aureomycin, and found it without any appreciable effect. Although a direct metabolic effect of aureomycin could not be definitely excluded, and it still remains a possibility, we were inclined to favor the third possibility, *i.e.*, the interaction between aureomycin and intestinal flora. It has been previously pointed out (1) that the temporary protection exerted by aureomycin, in contrast to the permanent protection achieved by the sulfur-containing amino acids, or vitamin E, tallies especially well with the assumption that the effect of aureomycin is mediated through the intestinal flora. One can consider that aureomycin acts through suppression of the intestinal flora, or at least of some of its constituents, and thus prevents the formation of bacterial metabolites with which the liver, in the absence of vitamin E or of sulfur-containing amino acids as "detoxifying agents," is unable to cope. If this assumption is correct, one can expect that such an effect will slowly wear off and that organisms may reappear in the intestinal flora which are resistant to aureomycin.

If aureomycin acts, albeit temporarily, *via* suppression of the intestinal flora, some of the other known antimicrobial agents should exert a comparable delaying effect on the production of massive hepatic necrosis. Penicillin, polymyxin, and chloromycetin were inactive, sulfaguanidine showed only slight indication of a positive effect, whereas streptomycin and terramycin—in increasing order—were definitely effective but not as beneficial as aureomycin.

For all practical purposes, ingested streptomycin is not absorbed from the intestinal tract. Thus, its beneficial effect further suggests suppression of the intestinal flora, as the mode of action. Combination with pectin increases the sterilizing effect of streptomycin in the intestine (10). Such combination has been found more effective than streptomycin alone in delaying massive hepatic necrosis (Table II).

If we assume that antimicrobial agents act *via* suppression of the intestinal flora, then the quantitative differences in their activity may be due either to differences in their ability to suppress those constituents of the intestinal flora which are essential for the production of hepatic necrosis, or to corresponding variations in the development of drug-resistant strains.

The nutritional effect of antimicrobial agents, when added to the necrogenic diet, was not limited to the delayed appearance of hepatic necrosis, but it manifested itself also in promotion of growth, especially during the first weeks of the experiments. This gain in weight was obtained not only with aureomycin (1), but also with all other antimicrobial agents, irrespective of their effect on hepatic necrosis. These findings are in accord with observations on growth promotion by succinylsulfathiazole and streptomycin (11), by phenylarsonic acid derivatives (12), and with the more recent reports on growth stimulation by aureomycin and the so called animal protein factor, containing B_{12} and aureomycin (13). In general, in the experiments carried out on chicks, turkeys, rats, and pigs, aureomycin appeared to be more potent than the other antimicrobial agents. At present, in the absence of exact bacteriological information regarding the effect of the various antimicrobial agents on the intestinal flora, it is impossible to explain the discrepancy between their effect on hepatic necrosis and on growth.

It is equally difficult to attribute the beneficial effect of aureomycin on hepatic necrosis and on hepatic cirrhosis to one common factor. For hepatic necrosis the possibility was discussed that in the absence of vitamin E or cystine as detoxifying agents, metabolites of the intestinal flora may injure the hepatic parenchyma. Inasmuch as cirrhosis develops in the presence of vitamin E and cystine (5) these detoxifying agents should be available in sufficient amount to prevent hepatic injury by metabolites of the intestinal flora. The fact that aureomycin still will prevent the development of hepatic cirrhosis, presents various possibilities:—

1. The intestinal flora might play a role in the production of cirrhosis; however, the "toxic" metabolites in question must be different from those supposedly instrumental in the production of necrosis and are not detoxified by vitamin E or cystine.

2. Aureomycin may act systemically, perhaps through the endocrine system or through direct metabolic reactions. In this connection it should be pointed out that in our experiment rats receiving aureomycin ate more and gained

more weight than the control animals. There was a slight but recognizable difference in the thyroid glands between the control animals and the rats receiving supplements of aureomycin, with hypertrophy and hyperplasia more marked in the animals receiving aureomycin. This is especially noteworthy in view of the prevention of dietary cirrhosis of the liver by goitrogenic substances (14).

3. Finally, the possibility of a "contaminant" present in aureomycin and acting as an anticirrhotic agent still cannot be excluded. For the elucidation of the effect of aureomycin in cirrhosis, further experiments, including paired-feeding experiments, are required.

The observations of Opie (15) on the intensification and modification of toxic necrosis and cirrhosis of the liver by intravenously administered cultures of *E. coli* or streptococcus may have a bearing on the findings here reported.

During recent years pancreatic changes accompanying dietary cirrhosis have been described by several authors (16). In the present experiments, in rats fed the cirrhosis-producing basal diet for 100 days, the pancreas appeared to be histologically normal despite the severe cirrhosis of the liver. The absence of pancreatic changes may be due to the relatively short duration of our experiment. If this explains such absence of pancreatic changes, the presented findings would indicate that cirrhosis precedes pancreatic changes, at least under the experimental conditions chosen.

The beneficial effect of aureomycin on rats kept on a cirrhosis-producing diet manifested itself in the prevention not only of cirrhosis of the liver but also of renal changes (5).

SUMMARY

The effect of various antimicrobial agents, such as aureomycin, terramycin, streptomycin, chloromycetin, penicillin, polymyxin, and sulfaguanidine on the development of massive dietary necrosis of the liver in rats has been studied. Delay in the production of hepatic necrosis was obtained from aureomycin and, to a lesser extent, from terramycin and streptomycin. Indication of temporary protection was shown by sulfaguanidine, whereas chloromycetin, polymyxin, and penicillin were not protective.

B_{12}, added alone, or in combination with aureomycin, to the basal experimental diet had no influence on the development of hepatic necrosis. A combination of pectin with streptomycin enhanced the protective effect of the antibiotic.

All the antimicrobial agents tested, without relation to their effect on hepatic necrosis, produced temporary stimulation of growth in the experimental animals.

The beneficial effect of aureomycin was not limited to the delay of hepatic necrosis but manifested itself also in the prevention of hepatic cirrhosis in rats fed a low protein (casein)–high fat diet. In contrast to control animals show-

ing the usual combination of cirrhosis and renal changes, the rats receiving supplements of aureomycin were free of both cirrhosis and renal changes. The rats receiving aureomycin took more food in and gained weight.

No microscopic alterations were seen in the pancreas of the control rats with cirrhosis.

In both groups of experiments (necrosis and cirrhosis) the antimicrobial agents, with the exception of penicillin, were given mixed with the food. Their possible effect on the intestinal flora is discussed.

BIBLIOGRAPHY

1. György, P., Stokes, J., Jr., Smith, W. H., and Goldblatt, H., *Am. J. Med. Sc.*, 1950, **220,** 6.
2. György, P., and Goldblatt, H., *J. Exp. Med.*, 1939, **70,** 355. Himsworth, H. P., and Glynn, L. E., *Clin. Sc.*, 1944, **5,** 133.
3. Dearing, W. H., and Heilman, F. R., *Proc. Staff Mayo Clin.*, 1950, **25,** 87.
4. Sborov, V. M., Jay, A. R., and Watson, C. J., *Proc. Centr. Soc. Clin. Research,* 1949, **22,** 70.
5. György, P., and Goldblatt, H., *J. Exp. Med.*, 1949, **89,** 245.
6. György, P., Rose, C. S., Tomarelli, R. M., and Goldblatt, H., *J. Nutrition*, 1950, **41,** 265.
7. Himsworth, H. P., and Lindan, O., *Nature*, 1949, **163,** 30.
8. György, P., and Rose, C. S., *Proc. Soc. Exp. Biol. and Med.*, 1950, **73,** 372.
9. Himsworth, H. P., The Liver and Its Diseases, Oxford, Blackwell and Co., 1947.
10. Wooldridge, W. E., and Mast, G. W., *Am. J. Surg.*, 1949, **78,** 881. Pulaski, E. J., Connell, J. F., and Seeley, S. F., *Ann. Surg.*, 1950, **132,** 225. Welch, H., Randall, W. A., and Price, C. W., *J. Am. Pharm. Assn. (Sc. edition)*, 1950, **39,** 486.
11. Moore, P. R., Evenson, A., Luckey, T. D., McCoy, E., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1946, **165,** 437.
12. Moorehouse, N. F., and Mayfield, O. J., *J. Parasitol.*, 1946, **32,** 20. Morehouse, N. F., *Poultry Sc.*, 1949, **28,** 375. Bird, H. R., Groschke, A. C., and Rubin, M., *J. Nutrition*, 1949, **37,** 215.
13. Stokstad, E. L. R., and Jukes, T. H., *Proc. Soc. Exp. Biol. and Med.*, 1950, **73,** 523. Luecke, R. W., McMillen, W. N., and Thorp, F., Jr., *Arch. Biochem.*, 1950, **26,** 326. Whitehill, A. R., Oleson, J. J., and Hutchings, B. L., *Proc. Soc. Exp. Biol. and Med.*, 1950, **74,** 11.
14. György, P., and Goldblatt, H., *Science*, 1945, **102,** 451. György, P., Rose, C. S., and Goldblatt, H., *Proc. Soc. Exp. Biol. and Med.*, 1948, **67,** 67.
15. Opie, E. L., *J. Exp. Med.*, 1910, **12,** 367.
16. Grossman, M. I., Greengard, H., and Ivy, A. C., *Am. J. Physiol.*, 1943, **138,** 676. Gillman, T., and Gillman, J., *Arch. Int. Med.*, 1945, **76,** 63. Lindsay, S., Entenmann, C., and Chaikoff, I. L., *Arch. Path.*, 1948, **45,** 1. Davies, J. N. P., *Lancet*, 1948, **254,** 317. Véghelyi, P. V., *Lancet*, 1948, **254,** 497. Waterlow, J., *Lancet*, 1948, **254,** 576. Goldberg, R. C., Chaikoff, I. L., and Dodge, A. H., *Proc. Soc. Soc. Exp. Biol. and Med.*, 1950, **74,** 869.