TABLE II.
Curative Effect of Aureomycin in Polyarthritis of Rats.

	No. of animals*	Incidence of arthritis: Pretreatment, %	Incidence of arthritis: 4 days after aureomycin, %	Avg arthrogram score: Pretreatment	Avg arthrogram score: 4 days after aureomycin
100 mg aureomycin per kg subcutaneously on 7th and 8th days after infection	20	55	5	1.6	0.15
Untreated controls	20	66	73	1.3	1.35

* The average body weight in both groups was 75 g.

tration of the drug. The untreated controls showed no measurable level of the antibiotic.

Preliminary clinical trials were made in 4 advanced cases of rheumatoid arthritis which had responded unsatisfactorily to several therapeutic agents, and one early case using 2 g aureomycin daily by mouth for 1 month. Two patients noted an improvement in appetite, and one patient had to discontinue the medication on the third day due to a marked gastrointestinal upset. None of these cases showed any improvement in range of motion of affected joints and 3 of them developed increased pain and swelling while under treatment. One case of Reiter's syndrome responded dramatically, gaining 6 lb during the first week of treatment and losing all joint pain. At the end of one month he showed no more joint swelling. His blood sedimentation rate (Wintrobe Method) decreased from 40 mm to 12 mm per hour in 2 weeks.

Summary. 1. The addition of aureomycin to the diet, subcutaneous administration, and gastric intubation of the antibiotic prevented and cured experimental polyarthritis of rats due to the L_4 strain of pleuropneumonia-like organism.

2. *In vitro* aureomycin prevented growth of the microbe in broth.

3. In preliminary clinical trials, several patients with chronic rheumatoid arthritis who responded unsatisfactorily to several therapeutic agents also failed to respond to aureomycin.

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17280. Effect of Incubation on the Cholesterol Partition in Human Serum.*

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Sperry[1] reported that the incubation of blood serum or plasma from normal human subjects resulted in a decrease in the amount of free cholesterol present without a change in the total cholesterol. It was concluded that esterification of some of the free cholesterol had taken place and that this had come about through the action of an enzyme, as the process was abolished by heating the serum to 55-60° prior to incubation. If this esterification of cholesterol *in vitro* were the result of enzymatic activity, it seemed possible that the enzyme might be absent or present in reduced amounts in the peripheral blood of patients with an abnormally low cholesterol ester fraction in the serum. This would

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1 Sperry, W. M., *J. Biol. Chem.*, 1935, **111**, 467.

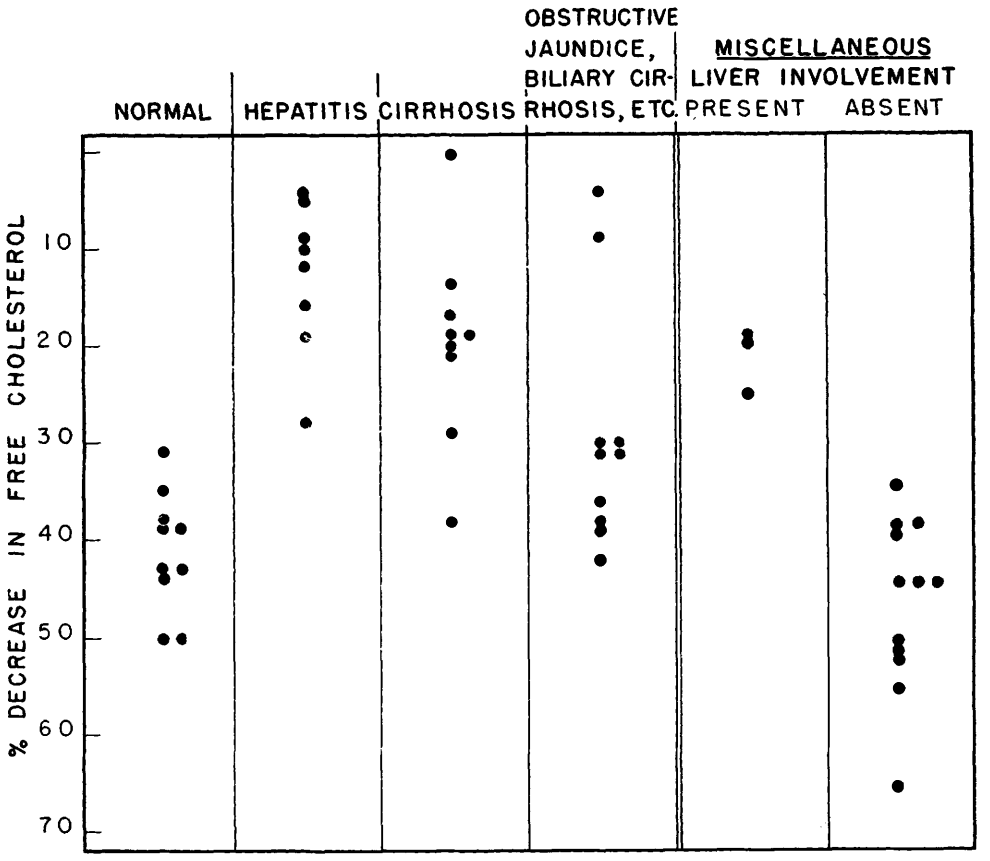

FIG. 1.
The percentage reduction in the amount of free cholesterol in the serum after incubation at 37° for 24 hours. Each dot represents one patient.

occur primarily in liver disease. To test this hypothesis the present investigation was undertaken.

Experimental. The effect of incubation on the serum cholesterol partition was studied in 10 normal individuals, 30 patients with liver disease, and 12 patients with a variety of disease conditions but without liver involvement. The concentration of total and free cholesterol in each sample of serum was determined by the method of Schoenheimer and Sperry[2] before and after incubation at 37° for 24 hours. The difference between the total and free cholesterol was assumed to represent the esterified cholesterol. Blood samples were usually obtained in the fasting state. No preservative was added to the serum before incubation. Hemolyzed samples were discarded as Sperry had found that hemolysis inhibits the reaction, and we had confirmed this observation.

The values obtained for the total cholesterol in each specimen before and after incubation were mostly within the limits of error of the method. In other words, the total cholesterol did not change. The decrease in the amount of free cholesterol with incubation varied from none to 69%. The results are summarized in Fig. 1.

In a number of instances the period of incubation was extended to 48 or 72 hours. This seemed to provide little added information even though a further decrease was observed in the amount of free cholesterol, and consequently the 24-hour period was adopted as standard procedure. In one case the decrease

[2] Schoenheimer, R., and Sperry, W. M., *J. Biol. Chem.*, 1934, **106**, 745.

TABLE I.
Effect of Heating Serum in Preventing a Decrease in Free Cholesterol with Incubation.

		Serum cholesterol				
			Free		Ester	
		Total mg %	mg %	% decrease	mg %	%
Subject 8	Control	268	75		193	72
	Incubated*	267	76	0	191	72
	" †	268	49	35	219	81
Subject 9	Control	180	47		133	74
	Incubated*	181	47	0	134	75
	" †	181	27	43	154	85

* After heating to 56° for one-half hour.
† Unheated.

in free cholesterol was determined after incubation for 3, 6, 12 and 24 hours. At 3 hours the drop amounted to 13%; at 6 hours it was 18%; at 12 hours, 32% and at 24 hours 42%.

We have confirmed Sperry's observation[1] that heating the serum to 56° before incubation prevents the drop in free cholesterol from taking place, presumably by destroying the enzyme responsible for the reaction. Data on the effect of preliminary heating on the serums of 2 normal subjects are shown in Table I.

Results. The decrease in the free cholesterol in the serums of 10 normal individuals ranged from 31% to 50% following incubation. Accordingly, decreases of more than 30% in 24 hours are considered normal, and a decrease of less than 30% is considered abnormal. This agrees with the results of Sperry[1] who, in 30 samples from 22 healthy young adults, obtained a decrease in free cholesterol of 29 to 84% after incubation for 24 to 72 hours.

Among the 30 patients with liver disease there were 8 with acute hepatitis. In all of these the drop in free cholesterol with incubation was less than normal, suggesting that the amount of enzyme present was decreased in this disorder. The decrease in free cholesterol ranged from 4% to 19% in 7, and was 28% in the remaining case. All of the patients in this group showed a low ester cholesterol (25 to 50%) prior to incubation. The serum bilirubin was elevated in all. The cephalin flocculation test was positive in 4, negative in 4. The alkaline phosphatase exceeded 5 Bodansky units in 5. In 2 patients the serum albumin was less than 4.0 g%. In general, there did not seem to be a close correlation between these other tests and the degree of reduction of free cholesterol on incubation.

In 8 of 9 patients with Laennec's cirrhosis of the liver, the decrease in free cholesterol with incubation was less than the empirical normal, ranging from none to 29%. The proportion of esterified cholesterol before incubation was slightly below normal (60 to 69%) in 7, and low (18%) in one. The serum bilirubin was increased in 7. The cephalin flocculation test was positive and the serum albumin reduced also in 7 cases. The alkaline phosphatase was between 5 and 6 Bodansky units in 3; less than 5 units in 5. The ninth patient in the group was admitted to the hospital because of bleeding esophageal varices, and was considered to have inactive cirrhosis. The serum of this patient showed a normal drop of 38% in free cholesterol after incubation, and all other chemical tests were normal as well.

In another group were 10 patients with obstructive jaundice, biliary cirrhosis, or extensive metastatic carcinoma of the liver. A characteristic of the group was an elevated alkaline phosphatase exceeding 10 Bodansky units. A reduction in free cholesterol following incubation that was interpreted as being within the normal or low normal range occurred in 8 of the 10 cases. The proportion of esterified cholesterol before incubation was less than 70% in 9 of these 10 cases. The

serum bilirubin was elevated in 7, the cephalin flocculation test was positive in one, abnormal A/G ratios were present in 2.

In a final group among the examples of liver disease there were 3 patients with miscellaneous disorders. One patient had disseminated lupus with ascites, a marked reduction in serum albumin, and a positive cephalin flocculation. Another was admitted in severe congestive heart failure with clinical jaundice and was found to have an elevated serum bilirubin, positive cephalin flocculation, and 50% bromsulfalein retention. The third patient showed extreme involvement of the liver by Hodgkin's disease at autopsy. The decrease in free cholesterol following incubation of serum from these 3 patients was interpreted as less than normal in all—19%, 25% and 20%.

As a further control, 12 patients with various diseases but without demonstrable liver involvement were studied. The group included 4 cases of anemia, 2 cases of fever of unknown origin, and single examples of optic neuritis, leukemia, chronic glomerulonephritis, diabetes, ichthyosis, and psoriasis. In all of these patients the reduction of the free cholesterol with incubation was considered normal. The decrease ranged from 35% to 69%. In 8 of these cases the proportion of esterified cholesterol before incubation was above 70%; in 4 it was between 58% and 69%.

Two or 3 determinations of the reduction of free cholesterol have been made at varying intervals on the serums of each of 6 patients. In a diabetic the decrease was constant on 3 occasions within 8 days. In two patients with infectious hepatitis who showed a decreased reduction in the amount of free cholesterol originally, the test returned to within normal limits with clinical improvement; in a third patient with this disease there was a slight progressive decline in the reduction of free cholesterol until the death of the patient. A patient with cirrhosis, who at first had an abnormally slight decrease in the amount of free cholesterol with incubation, later showed a reduction considered within the normal range. This was accompanied by very little change in clinical condition and chemically only by a rise in serum albumin from 2.7 g to 3.3 g. A patient with heart failure and clinical jaundice, who at first had a reduction of 25% in free cholesterol with incubation showed a normal reduction of 46% after clinical improvement and disappearance of the jaundice.

Discussion. The mechanism of the reaction described is not clear. An enzyme appears to be involved in the reduction in the amount of free cholesterol in the serum following incubation. This enzyme effect is decreased when the liver parenchyma is involved as in hepatitis and cirrhosis, and also occasionally in obstructive jaundice. Work is in progress in an attempt to elucidate the mechanism and to establish the clinical significance of the reaction here described.

The decrease in free cholesterol with incubation does not parallel the rise in serum bilirubin. Sperry[3] found that the addition of bile salts to the serum *in vitro* inhibited the reaction, but we have found a normal reduction in free cholesterol in the presence of clinical jaundice and a decreased reduction without an increase in the serum bilirubin. There is certainly no correlation between this test and the cephalin flocculation test, alkaline phosphatase or serum albumin. There seems to be a somewhat better correlation with the proportion of esterified cholesterol in the serum before incubation, but even in this there are striking exceptions.

While the change in free cholesterol has been given in percentages, it should be pointed out that it may eventually prove desirable to express this in milligrams per cent. Decision on this point must await further information from work now in progress on the nature of the enzyme and its action.

Summary. Incubation of normal human serum and that of patients with disease not involving the liver results in a drop in free cholesterol of more than 30% without change in the total cholesterol. The reaction is probably due to an enzyme, as the decrease does not occur when the serum is heated to 56° before incubation.

[3] Sperry, W. M., and Stoyanoff, V. A., *J. Biol. Chem.*, 1937, **117**, 525.

In this small series, the decrease in free cholesterol with incubation was usually less than normal with disease of the liver parenchyma and occasionally with extra-hepatic obstructive jaundice. The decrease in free cholesterol apparently did not parallel changes in serum bilirubin, alkaline phosphatase, serum albumin, or the cephalin flocculation test. There appeared to be some correlation with the percentage of cholesterol esters in the control serum.

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17281. Effect of Antihistamine on the Localization of Trypan Blue in Xylene Treated Areas of Skin.

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Some data and experimental observations have suggested that histamine may play a fundamental role in the development of local areas of inflammation.[1-3] In support of this, it has been shown that trypan blue following an intravenous injection localizes and concentrates in areas of skin previously injected with histamine.[4] The effects of antihistamine drugs on capillary permeability have been reviewed recently by Last and Loew.[5] These investigators found that the localization of trypan blue in areas of skin previously injected with different chemical and biological preparations was not modified by the intravenous injection of the antihistamine preparation "Benadryl".[5]

The present experiments were performed to study the effect of two of the more recent antihistamine preparations on the hyperemia and the localization and concentration of trypan blue in areas of skin treated with xylene.

Methods and materials. Fifteen rabbits were used. Their hair was carefully removed 24 to 48 hours preceding the time of the experiment. Two antihistamine preparations were used: Pyrrlidineethyl-phenothiazine hydrochloride (Pyrrolazote*); and, N,N-Dimethylly-N′ (alpha-pyridyl)-N′ (alpha-thenyl)-ethylenediamine hydrochloride (Thenylene†). A solution of each containing 10 mg per cc was made in physiologic sodium chloride. The injections of Pyrrolazote were made intravenously, while Thenylene was injected both intravenously and intraperitoneally. Ten cc of a 0.2% solution of trypan blue was injected intravenously. Xylene was carefully applied with a cotton applicator to local areas of skin at intervals varying from one to 140 minutes during the time that the rabbits were under the influence of the antihistamine preparations and before the dye was injected intravenously. The xylene treated areas were carefully observed during the development of hyperemia and during the time of the localization and concentration of the dye. These observations extended over a period of 2 hours.

In 8 rabbits 0.2 cc of the antihistamine preparations was injected intradermally from immediately to 60 minutes preceding the time of the intravenous injection of trypan blue. An equal volume of both a physiologic sodium chloride solution and distilled water was used as controls.

1 Lewis, Sir Thomas, London, Shaw and Shaw, 1927.

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3 Mayer, R. L., *Ann. Allergy*, 1947, **5**, 113.

4 Rigdon, R. H., *J. Lab. and Clin. Med.*, 1942, **27**, 1554.

5 Last, M. R., and Loew, E. R., *J. Pharm. and Exp. Therap.*, 1947, **89**, 81.

* Obtained from the Upjohn Company, Kalamazoo, Mich.

† Obtained from the Abbot Research Laboratories, Chicago, Ill.