served that the contractions of the ileum began almost immediately on the addition of the choline and were constant after a relatively few contractions. If it were necessary to acetylate the choline in order for it to be active one might expect a delay followed by a relatively long period of increasing contractions. Second, by analogy to studies of the action of choline and related compounds on the heart of *Venus mercenaria*(8. 9) it seems also that the first possibility is more likely. It thus appears that our results are best explained by assuming that choline and certain other compounds related to it are capable *per se* of stimulating intestinal muscle. The mechanism of this action may be similar to the coenzyme theory proposed by Welsh(10) to explain the mode of action of acetylcholine. Accordingly, triethylcholine, β-methyltriethylcholine and α-methyl- α-hydroxymethylcholine have anticholine activity because they compete with choline for active sites on the apoenzyme.

*Summary.* Choline-like and anticholine activities of a series of compounds structurally related to choline have been determined using segments of rabbit ileum. Triethylcholine, β-methyltriethylcholine and α-methyl- α-hydroxymethylcholine were competitive inhibitors of choline in this tissue but had choline-like activity on segments of jejunum.

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## Effect of Aspirin Administration on Serum Glutamic Oxaloacetic and Glutamic Pyruvic Transaminases in Children.* (22671)

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Elevations of the serum glutamic oxaloacetic transaminase (SGO-T) activity during the course of rheumatic fever in children have been noted previously(1). Despite the irregular pattern of these elevations and the absence of parallel alterations in the indices of inflammation commonly used (erythrocyte sedimentation rate, white blood cell count and C-reactive protein) and in the clinical course of the patients, it was suggested that the alterations of SGO-T were the result of myocardial necrosis during the course of rheumatic carditis. This enzyme is known to be present in the heart in high concentration(2) and elevation of its activity in the serum following myocardial infarction in man(3) and in dogs (4) has been described. Elevations of SGO-T also occur in human(5) and murine(6) hepatic diseases, but no clinical evidence of hepatic disease was present in the rheumatic fever patients studied and their liver function tests were normal(1). Drugs such as digitalis and mercurial diuretics which are often used in the treatment of patients with rheumatic fever were shown to have no effect on

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the SGO-T(3). Moreover, no alteration of SGO-T was noted during the administration of 4 to 6 g of aspirin daily for one week to a group of non-rheumatic adults(1).

Despite this latter observation a possible effect of aspirin upon the serum activity of glutamic oxaloacetic transaminase in children was suspected as a larger series of children with rheumatic fever was subsequently studied. The present investigation was undertaken to explore this relationship. The activity of another enzyme, glutamic pyruvic transaminase (SGP-T) was studied in the sera of a group of patients in an attempt to determine whether serum activity of another transaminase in addition to SGO-T was affected by aspirin administration. In addition, since glutamic pyruvic transaminase is found in the liver in 6 times the concentration found in the heart(7), it was hoped that the source of the increased serum concentration of SGO-T might be suggested by the results of simultaneous determinations of the two transaminases. It has, in fact, been reported, in contrast to the findings with SGO-T, that the serum activity of glutamic pyruvic transaminase (SGP-T) is increased only slightly following myocardial necrosis in man. Similar elevations of the serum activities of both enzymes are found in liver diseases(7).

*Materials and methods.* Twenty-three children from the wards of Irvington House, a hospital and convalescent home for children with rheumatic fever, were studied. Their ages ranged from 5 to 15.

Group 1: Of the 14 children included in this group 12 received aspirin (acetyl salicylic acid) and 2 sodium salicylate. None was considered to have definite evidence of active rheumatic fever: 7 had questionable disease activity and 7 had no evidence of rheumatic activity by all clinical and laboratory criteria. Aspirin and sodium salicylate were administered in daily dosages of 0.6 to 1 g per 15 lb of body weight for a minimum of 4 weeks. The SGO-T was measured during a short control period and then twice weekly during the period of salicylate administration.

Group 2: The second group selected for study of both SGO-T and SGP-T consisted of 9 children, 7 of whom had no clinical or laboratory evidence of rheumatic activity. One patient was studied during a mild clinical rebound of rheumatic fever. The ninth patient had acute rheumatic polyarthritis with no evidence of carditis. Aspirin was administered in a daily dose of 0.6 g per 15 lb of body weight for an average period of 16 days. The dose was then increased to 1 g per 15 lb of body weight until symptoms and signs of toxicity occurred. At this time the aspirin was discontinued.

The SGO-T and SGP-T activities were determined twice weekly during a short control period and during the period of aspirin administration. The specimens of blood obtained were allowed to clot at room temperature. The sera were then separated immediately and frozen and the determinations of enzyme activity were performed within 3 days. Care was exercised to prevent hemolysis since this has been shown to increase the measured SGO-T activity(8). SGO-T activity was measured by the spectrophotometric method of Karmen *et al.*(8). SGP-T activity was measured by a similar method described by Wróblewski and LaDue(7). By these methods the normal range of activity of SGO-T is 10-40 u/ml/min. and of SGP-T is 5-35 u/ml/min. Rises of SGO-T to 50 units or more and of SGP-T to 45 units or higher are considered to be significant.

Blood salicylate levels were determined twice weekly using the method of Brodie, Udenfriend and Coburn(9). C-reactive protein was determined weekly by the capillary precipitin method described by Anderson and McCarty(10). Erythrocyte sedimentation rate was determined weekly by the Wintrobe method(11). To rule out the possibility of a direct effect of aspirin upon the rate of change of optical density of the reaction mixture in the spectrophotometric methods used, 2 series of experiments were done: A. Solutions of aspirin in phosphate buffer (pH 7.4) were added to the reduced coenzyme I to final concentrations of 10 to 60 mg % and the optical densities of the solutions measured for 10 minutes. B. Both sodium salicylate and aspirin were dissolved in normal serum to

TABLE I. Effect of Aspirin and Sodium Salicylate Administration on Serum Glutamic Oxaloacetic Transaminase Activity.

| Patient | Rheumatic activity | Drug administered | Maximal level of SGO-T (u/ml) |
|---|---|---|---|
| 1 | ? | Aspirin | 146 |
| 2 | ? | " | 88 |
| 3 | ? | " | 128 |
| 4 | No | " | 93 |
| 5 | ? | " | <50 |
| 6 | No | " | 132 |
| 7 | " | " | 76 |
| 8 | " | " | <50 |
| 9 | " | " | " |
| 10 | ? | " | " |
| 11 | ? | " | " |
| 12 | No | " | " |
| 13 | " | Sodium salicylate | 100 |
| 14 | ? | " " | <50 |

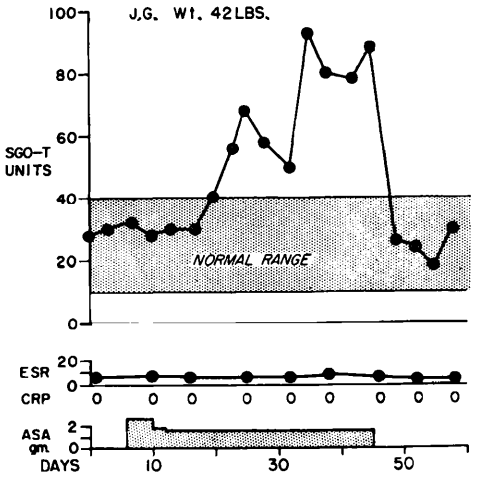


FIG. 1.

final concentrations of 5 to 40 mg %. The SGO-T and SGP-T activities of the sera were measured and compared with those of the same sera before the addition of these drugs.

*Results.* The effects of the administration of aspirin and sodium salicylate on serum transaminase activities in the patients of the 2 groups studied are summarized in Tables I and II. All patients had normal enzyme activities in their sera in the control period, before the administration of aspirin or of sodium salicylate. It is apparent that of 12 children in the first group receiving aspirin, 6 showed elevations of SGO-T to 50 units or greater. The maximal rise was to a level of 146 units. Three of these patients had no evidence of rheumatic activity and 3 were considered to have possible activity.

One of the 2 children receiving sodium salicylate showed an elevation of the SGO-T to 100 units. This patient did not have active rheumatic fever. The concentration of SGO-T in the other patient rose to 44 units, a borderline value.

A typical case is illustrated in Fig. 1. J.G., a patient convalescent from rheumatic fever and without evidence of rheumatic activity was maintained on aspirin, 1.2 to 2.4 g daily for more than one month. The SGO-transaminase first rose to an abnormal concentration 2 weeks after initiation of therapy, reaching a peak of 93 units 26 days after the first dose of aspirin. The SGO-T returned to a normal value within 2 days after discontinuation of the drug.

Table II shows the simultaneous variations of both SGO-T and SGP-T in the second group of 9 children in whom aspirin was administered until the appearance of toxic symptoms. Six of the children developed elevations of both SGO-T and SGP-T. Maximal levels of 540 SGO-T units and 900 SGP-T units were noted. These occurred in a patient who had had infectious hepatitis 18 months earlier. The shortest duration of aspirin administration resulting in significant rises of either enzyme was 6 days. Twenty-two days of continuous administration elapsed before the first elevation in patient No. 6.

TABLE II. Effect of Aspirin Administration on Serum Glutamic Oxaloacetic and Serum Glutamic Pyruvic Transaminases.

| Patient | Rheumatic activity | No. of days of aspirin admin. previous to 1st significant rise | | Maximal level | |
|---|---|---|---|---|---|
| | | SGO-T | SGP-T | SGO-T | SGP-T |
| 1* | No | 4 | 11 | 540 | 900 |
| 2 | " | 10 | 14 | 180 | 140 |
| 3 | " | 13 | 13 | 120 | 216 |
| 4 | " | no rise | no rise | <50 | <45 |
| 5 | " | 13 | 13 | 62 | 50 |
| 6 | " | 22 | 22 | 64 | 45 |
| 7 | " | no rise | no rise | <50 | <45 |
| 8 | ? | " | " | <50 | <45 |
| 9 | Yes | 6 | 6 | 74 | 76 |

* This patient suffered from infectious hepatitis 18 mo earlier.

whereas patient No. 7 received aspirin for 32 days with no detectable rise in the serum activity of either transaminase, despite comparable dosages and serum salicylate levels. A second transitory rise of one or both enzymes was noted in 4 cases 3 to 10 days after cessation of aspirin therapy at a time when there was no detectable salicylate in the patients' sera (Fig. 2).

No consistent relationship between serum salicylate levels and serum enzyme activities was found. There was no evident correlation between changes in the indices of inflammation (erythrocyte sedimentation rate, white blood cell count and C-reactive protein) and SGO-T or SGP-T.

No change in the rate of the transamination reaction of the test solutions or of the optical density of reduced coenzyme I was produced by the *in vitro* addition of aspirin or of sodium salicylate. The concentrations of salicylate tested encompassed the range of concentrations measured in the sera of the patients studied.

*Discussion.* It is clear that aspirin administration can influence the serum activity of glutamic oxaloacetic and glutamic pyruvic transaminases in children. For this reason any increase in the serum activity of these enzymes during the course of rheumatic fever should be evaluated with great care when aspirin is employed in the therapy of these patients.

The mechanisms whereby this aspirin effect is mediated are unknown at the present time. A direct effect of aspirin upon the rate of the transamination reaction in the test mixture or upon the optical density of reduced coenzyme I is ruled out by the *in vitro* studies mentioned. Among the several other possible explanations the possibility of an increased release of these enzymes from the liver as a result of aspirin administration deserves consideration. Experimental and clinical studies have documented changes in hepatic function and morphology following aspirin administration(12). SGO-T has been shown to be a sensitive and early indicator of hepatic damage(5). Furthermore, the simultaneous elevation of SGO-T and SGP-T is consistent

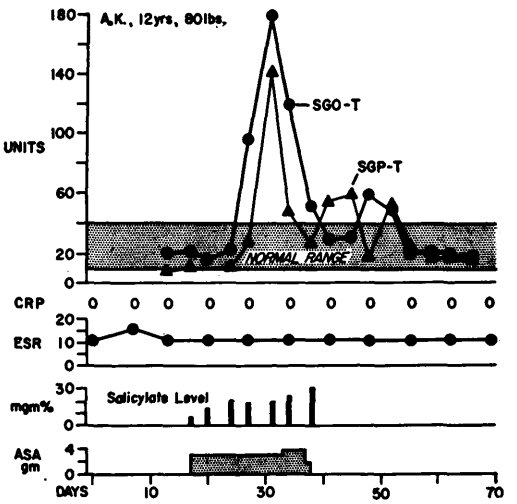


FIG. 2.

with the possibility that the liver is the source of the increase of the serum activity of these enzymes.

*Summary.* The effect of aspirin and of sodium salicylate administration on the serum glutamic oxaloacetic transaminase activity in 14 children and its effect on both the serum glutamic oxaloacetic transaminase and the serum glutamic pyruvic transaminase in an additional 9 children has been studied. Elevations to abnormal levels were observed in more than 50% of the cases. The importance of this finding in relation to the interpretation of the serum glutamic oxaloacetic transaminase elevations in rheumatic fever is pointed out and some possible underlying reasons for this phenomenon are mentioned.

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## Corn Oil and Hypercholesteremic Response in the Cholesterol-Fed Chick.* (22672)

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Recently Ahrens(1,2), Kinsell(3,4,5,6) and Bronte-Stewart(7) and their coworkers have reported unusual reductions of the serum cholesterol in subjects receiving a formula diet in which various vegetable oils, in rather large quantities, were the main source of fat. Ahrens, *et al.*,(2) demonstrated that corn oil was the most effective of these agents in maintaining a low level of serum cholesterol. They also have shown a correlation between the iodine number and the hypocholesteremic effect of several vegetable oils, and have postulated that the degree of unsaturation of the fatty acids is responsible for that effect. Meanwhile, we had noted a lower level of serum cholesterol in cholesterol-fed control chicks used to assay fractions of a hypocholesteremic brain extract(8). This difference could be related to the fact that we had begun to use corn oil instead of cottonseed oil as a vehicle for the administration of cholesterol in the atherogenic diet.

The following experiments were performed in an effort to test the hypothesis that it is the slightly higher level of unsaturated fatty acids in corn oil which distinguishes it from cottonseed oil in its effect on the cholesterol-fed bird.

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*Methods.* In each experiment, 40 8-week-old White Rock cockerels were divided into 4 dietary groups, each of which were offered equal weights of the diets. Diet consumption was estimated daily, body weight weekly. Two birds losing weight and apparently ill were excluded from the data. All of the diet offered was eaten, except as noted below. The chicks were bled from the alar vein at 2-week intervals, and the plasma analyzed for serum cholesterol by the method of Abell, *et al.*(9). At termination of the experiments, the aortae were examined for gross atherosclerotic plaques and graded as described by Horlick and Katz(10).

Experiment 1: To test the importance of the relative proportions of oleic and linoleic acids as they occur in corn oil as opposed to cottonseed oil, we used 2 "neo-fat" fatty acid products of Armour Chemical Division. Neo-Fat 105 consists of fatty acids redistilled from the cottonseed oil; Neo-Fat 110 is also derived from cottonseed oil fatty acids but fractionated so as to approximate the same relative proportions of palmitic, oleic and linoleic acids as occur in corn oil. According to the producers, these 2 distillates had the same minor difference in iodine number seen between corn oil and cottonseed oil, but neither had any myristic acid, which is normally present in corn oil. Each contained approximately 2% unsaponifiable matter derived from cottonseed oil. One group was fed 10% solvent extracted corn oil (Mazola); the second group 10% cottonseed oil (Puritan Oil, Procter and Gam-