TECHNICAL PAPERS

The Antirheumatic Effect of Sodium Gentisate

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The mechanisms involved in the activity of rheumatic diseases are as unknown as is the rationale of the antirheumatic action of salicylate. Salicylate administration has been shown to inhibit the spreading effect of hyaluronidase (*1*). *In vitro,* however, salicylate inhibits hyaluronidase in very high concentrations only, whereas the biological oxidation product of salicylate, gentisic acid, does so *in vitro* in concentrations of a few μg/ml (*3*). The inactivation of the enzyme is apparently irreversible and is believed to be due to a condensation of the semiquinone with the enzyme protein.

Since increased hyaluronidase activity has been suspected as a possible cause of the breakdown of interfibrillar cement in rheumatic diseases (*2*), the antirheumatic action of Na gentisate (supplied by Hoffmann-LaRoche, Inc., Nutley, New Jersey) has been investigated in a small number of patients. The results have been sufficiently uniform to warrant the present report. Gentisate has the same antirheumatic effect as salicylate without some of its disadvantages. In 5 patients with acute rheumatic fever, the administration of Na gentisate in doses comparable to those customarily employed for salicylate has been followed by disappearance of pain, swelling, and heat in the joints, by the fall of temperature to normal, and by fall in sedimentation rate. In one patient, withdrawal of gentisate after 3 days of administration was followed within 44 hrs by a return of acute joint symptoms, which again responded promptly to renewed administration of gentisate. The joint pain of 7 patients with rheumatoid arthritis has responded similarly to gentisate as to equivalent amounts of salicylate. In one patient, the salicylate was not tolerated because of the co-existence of a chronic duodenal ulcer, whereas gentisate caused no gastric irritation. Four patients with persistently active rheumatic fever—so-called "chronic rheumatic fever"—have responded similarly to gentisate and salicylate.

No untoward effects have been observed in the patients given as much as 10 gm/day, save in one patient who, on 8 gm/day, developed some epigastric distress which subsided immediately on withdrawal of the gentisate. No significant increase in prothrombin time, no tinnitus or aural symptoms have developed. No sign of methemoglobinemia or of liver damage has been observed. It seems significant that the increase in urinary glucuronic acid excretion observed with salicylate ingestion (*4*) does not occur with gentisate.

Only about one-quarter of the gentisate ingested was recovered in the urine as gentisic acid. So far we have been unable to detect gentisic acid in the blood, using a color method by which 5 γ/cc of hydroquinone in the urine can be detected. It appears that gentisate is rapidly oxidized in the body.

In summary, sodium gentisate appears to exert antirheumatic activity equal to, or greater than, that of salicylate. It is suggested that the antirheumatic action of salicylate is due to its oxidation product, gentisate. A detailed report of this work will be published shortly.

[1] Supported in part by the Josiah Macy, Jr. Foundation.

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Does Glutamic Acid Have Any Effect on Learning?

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Recent studies of the role of glutamic acid in learning and intellectual capacities have presented a picture of conflicting findings. Following a clinical report (*6*) that the feeding of excess glutamic acid to epileptic children seemed to improve their 'mental alertness,' Zimmerman and Ross (*9*) did a controlled study on the learning ability of albino rats of the Sherman strain. They found that feeding either proline or glutamic acid in 200-mg doses in excess of a basic chick Growena diet resulted in a superior performance of these animals on a Warner-Warden, 8-cul, single alternation maze. Later, Albert and Warden (*2*) reported that excess glutamic acid had beneficial effects on the performance of rats in a complex reasoning problem. Furthermore, extension of this work to humans in studies of feebleminded children has suggested that excess glutamic acid intake can increase the IQ as measured by standard intelligence tests (*1, 7, 8*). Since the earlier animal studies, however, two reports have appeared which failed to demonstrate any effects of glutamic acid on the rate of learning or in the reasoning ability of the albino rat. Marx (*5*) found no difference between glutamic-fed and control animals in the learning of a Stone multiple-T water maze. Hamilton and Maher (*3*) reported that glutamic acid feeding did not result

in a higher level of performance of rats on the Maier three-table test of reasoning.

These negative results, however, cannot be taken as disproof of the positive effect of glutamic acid which has been reported, for in each experiment there were differences in procedure with respect to a number of important variables: basic diet used to maintain experimental and control animals, amount of excess glutamic acid administered daily, portion of the life span covered by experimental feeding, age and strain of animals, and type of learning or reasoning tests used. At best, the negative findings indicate that beneficial effects of glutamic acid administration are not general to all procedures, strains, dosages, ages, and tests or some combination of these.

TABLE 1

MEAN PERFORMANCE ON WARNER-WARDEN SINGLE ALTERNATION MAZE (8 CULS)

	N	No. of trials	Trials to meet criterion		Total errors		Total time (sec)	
			M	σ	M	σ	M	σ
Control	14	21	5.1	4.0	11.4	4.5	663	568
Glutamic	14	21	5.1	2.3	14.2	4.8	597	287

It was decided, therefore, to duplicate, as nearly as possible, the most clear-cut of the positive findings with glutamic acid in order to demonstrate clearly, its proper role in learning ability. To this end, the procedures of Zimmerman and Ross were followed in every detail except for the strain of animals used. Twenty-eight pigmented rats from the Johns Hopkins colony (descendants of the Lashley strain) were divided into two groups of 14 animals each, matched for sex, weight, and litter. At the age of 6 weeks, all animals were taken off the colony diet of Purina Fox Chow checkers, which they had had *ad libitum*, and were placed on a 24-hr feeding schedule in which they had access to a Growena chick mash for 1 hr a day in individual feeding cages. Before each hour of daily feeding, the experimental animals were given a 5-gm dish of the basic diet, containing 200 mg of neutralized 1 (+) glutamic acid. The control animals had the extra 5-gm meal but never received any glutamic acid supplement. This feeding procedure was maintained throughout the entire experiment. All animals were weighed each day before feeding.

After 10 days of glutamic acid feeding, both control and experimental rats were allowed daily sessions of preliminary exploration of the disconnected maze units for 4 days. At the end of this time, each rat was given one trial daily in the connected maze for 21 days. The order in which animals were tested each day was maintained throughout the experiment. Furthermore, to avoid artifacts of procedure, experimental and control animals were alternated on the maze runs throughout each session.

The results of this experimental duplication of the study of Zimmerman and Ross indicated *no difference in the maze performance of control and glutamic-fed animals* (Table 1). Statistical analysis indicates that the total time and error scores and the number of trials required to reach a criterion of 4 out of 5 errorless runs were essentially the same in each group. In fact, both groups of animals learned the maze about as fast as Zimmerman and Ross's glutamic-fed animals. Growth curves for each group of rats were virtually identical.

Since it appeared that the Warner-Warden maze might be too easy a test to differentiate between normal and glutamic-fed animals of our strain, the rats were continued on the experimental feeding regime and were subsequently tested on two mazes of increasing difficulty: the Warner-Warden double alternation maze (8 culs) and a 4-cul, double alternation, elevated maze used by Hunter (*4*). In the former test, smell cues were eliminated by spraying the maze with cresol between the runs of individual animals. In the latter test, the units of the maze were rotated and interchanged between trials, but the animals were not blinded.

TABLE 2

MEAN PERFORMANCE ON WARNER-WARDEN DOUBLE ALTERNATION MAZE (8 CULS)

	N	No. of trials	Trials to meet criterion		Total errors		Total time (sec)	
			M	σ	M	σ	M	σ
Control	14	11	5.4	3.3	13.4	5.9	514	331
Glutamic	14	11	5.9	2.3	15.1	3.6	338	110

TABLE 3

MEAN PERFORMANCE ON ELEVATED DOUBLE ALTERNATION MAZE (4 CULS)

	N	No. of trials	Total errors		Total time (sec)	
			M	σ	M	σ
Control	14	24	35.5	3.4	513	113
Glutamic	14	24	35.6	6.6	458	142

Even in these more difficult tests, *no difference could be detected between experimental and control animals with respect to errors, time, or number of trials to reach criterion* (Tables 2 and 3).

In view of these findings it must be concluded that there is no effect of excess glutamic acid on the maze-learning ability of our strain of rats. It appears clear that the facilitating effects of glutamic acid on learning that have been reported are not general. Because of the controls we used, it is not likely that the positive results can be considered some function of the basic diet used, the age of the animals, the type of behavioral test employed, or the dosages of glutamic acid, although variation in any one of these variables could conceivably have affected results obtained in such an experiment. Strain differences remain as the only possible variable that could account for the difference in results between our experiment and that of Zimmerman and Ross. Other negative-

results with albino rats indicate that the strain difference is not a matter of whether the rats are albino or pigmented, but it is possible that the beneficial effects of glutamic acid might be specific to the Sherman strain of albino rats. At the present time experiments are being designed to test animals of the Sherman strain. Further experiments will be done in which proline and other compounds metabolically related to glutamic acid will be studied. At this point, however, it must be concluded that there is little evidence for a facilitating effect of excess glutamic acid feeding on the learning ability of the rat.

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Effect of Heparin and Dicoumarol on Sludge Formation

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Since Kniseley published his findings of the sludge phenomenon (*2*) in shock and certain other disease states it has been assumed that this condition was a precursor of thrombosis, as evidenced by the following quotation from a recent editorial in the *Journal of the American Medical Association* (*4*):

> As a result of publication of this report (Kniseley), many practicing physicians have suggested use of heparin or Dicoumarol to prevent the sludging of blood of patients met in their daily practices. Until these observations have been extensively checked by other investigators, introduction of new methods of treatment to combat sludging of blood should be highly experimental.

In the course of vascular occlusion experiments in dogs (*3*), we found that we could produce sludge at will in the smaller vessels distal to an occlusion within a relatively short time. Since our studies included an evaluation of various therapeutic agents in vascular occlusive states, we felt it necessary not only to determine the effects of anticoagulants on thrombus formation, but to investigate their effects on sludge formation as well.

Therefore, an experiment was set up to determine the effects of heparin and Dicoumarol on sludge formation in small vessels following acute main-stem venous occlusions. We used venous occlusions exclusively, since the process was usually more gradual than in the arterial occlusions and could be followed more closely.

All observations were made on mesenteric vessels, using a Kniseley fused quartz rod transillumination apparatus (*1*) employing a constant-temperature tissue bath with variable volume flow. Young, small dogs were used, and intravenous Nembutal anesthesia was employed. A special Lucite tray held the dog's mesentery in a nonstretched position, submersed in constantly circulating mammalian Ringer's solution at body temperature. The microscope was fixed on small vessels. A small precapillary artery and vein running side by side were chosen for observation, the artery measuring from .054 to .144 mm in the various animals and the vein from .090 to .288 mm. The capillaries stemming from such vessels were observed in the same microscopic field.

In over 70 main-stem venous occlusions done on normal dogs we had consistently noticed sludge formation in the small vessels within 10–20 min following occlusion. Six animals in this group, chosen at random, comprised our control series. A second group of 6 animals was used in a preliminary experiment in which heparin was given by intravenous injection into a tongue vein after sludge formation was noted. A third group of 6 animals was heparinized before venous occlusion, while a fourth group of 6 animals was dicoumarolized before venous occlusion.

Group I: Control. The portal vein or superior mesenteric vein was occluded in the usual manner by a rubber-tipped clamp which was allowed to remain in place for about 1 hr before release. As the stream slowed and anoxia progressed, sludge formation was noted in each instance within 10–20 min in the small peripheral mesenteric vessels. As the stream slowed further, thrombus formation was noted in some of the small vessels, especially in the capillaries and venules, usually within 30 min after the appearance of sludge. The groups of agglutinated cells traveled in spurts through the spastic artery, while in the vein they became attached sooner or later to the endothelial lining of the vessel. As a small group of cells became adherent to the side of the vessel, more cells became attached to the mass, and a thrombus formed which eventually obliterated the entire lumen as the stream stopped flowing. In many instances, if the occlusion was released at this stage, flow again resulted as the liquid stream washed the agglutinated particles through. Other small vessels maintained their thrombosis and did not become patent. For the most part, by the time of release (about 1 hr), the flow had either ceased completely or was in the ebbing stage within most small vessels. After release of the occlusion, the vessels not showing renewed motion of the stream were considered thrombosed.

The process of thrombosis, then, in the small vessels under microscopic observation appeared to consist of the following steps: (a) sludge formation, (b) adherence of sludged masses to the endothelial lining of the vessels, (c) the "piling up" of more cells to such an agglutinated mass, and (d) stoppage of flow after complete occlusion of the vessel by an agglutinated mass of cells.

Group II: Heparin administered after the appearance of sludge formation. Using dogs of about 5 kilos, he-