It is of interest, therefore, to speculate why the beneficial effect was not obtained with antibiotics other than aureomycin, since their antibacterial spectra are not too dissimilar. It may be that some of the antibiotics were rapidly destroyed in the intestinal tract or by body tissues and therefore could not be effective against the "deleterious" bacteria that were either consuming citrovorum factor or destroying it and therefore making it unavailable to the host. One of the greatest defects in our information at present is the fact that little is known about the synthetic abilities of a particular microorganism and less information is available on the interrelationships between the various organisms in the intestinal tract. The suppression of some bacteria and/or the resulting ability of others to flourish may account for the relative effectiveness of an antibiotic to indirectly provide the particular substance required by the host.

Summary. Streptomycin, terramycin, penicillin and chloromycetin, unlike aureomycin, were ineffective in overcoming the deficiency induced by the feeding of aminopterin to rats. Citrovorum factor is able to counteract aminopterin at low levels, indicating that the aminopterin may be a citrovorum factor antagonist as well as a folic acid antagonist. The addition of aureomycin to a diet containing citrovorum factor provides better growth than could be obtained on either supplement alone.

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Normal Mucoprotein Values in the Dog; Response to Distemper Vaccine and to Endocarditis.* (19434)

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The alterations in serum proteins in a variety of clinical conditions have received considerable attention in recent years. One field of investigation has concentrated on the mucoprotein constituents of the blood serum. It has been shown by several investigators (1-5) that an elevation in the mucoprotein concentration in the human occurs in many conditions. Winzler and coworkers(1,2) have demonstrated an increase in patients with malignancies of various types. They likewise showed that patients with pneumonia have an elevated mucoprotein level. Other infections, both of bacterial and of virus origin, are reported to be accompanied by a rise in mucoprotein values(3). It would appear that the mucoprotein component of the blood exhibits a non-specific response to body tissue injury. The only instance of reduction in the mucoprotein value clinically is that which occurs with severe renal disease(5). Recently, attention has been directed to the response of the mucoproteins in rheumatic fever(3,4,6). Adams and coworkers(6), on the basis of studies in rheumatic fever patients, have suggested that even though adrenocorticotropin therapy often produced an apparent clinical and laboratory remission, the abnormal mucoprotein elevations persisted in their patients

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until the rheumatic process became inactive.

The above mentioned studies have led to the investigation of serum mucoprotein levels in dogs under the following conditions: Group I, normal dogs; Group II, response to a distemper vaccine in normal dogs; Group III, dogs with a chronic cardiovascular stress in the form of large arteriovenous fistulas; Group IV, dogs with bacterial endocarditis produced by the intravenous injection of small numbers of bacteria following the construction of large arteriovenous shunts.

Methods of study. The analysis of the serum mucoproteins was carried out after the method of Winzler(1), using the Folin-Ciocalteau reagent, with adaptation to the Coleman Photoelectric Colorimeter. This method actually determines mucoprotein from an estimation of its tyrosine content, which has been found to bear a constant relationship to mucoprotein carbohydrate and nitrogen. *Group I. Normal dogs.* Blood for the determination of the normal mucoprotein control values was drawn, immediately upon admission to the animal colony, from each of 55 mongrel dogs, unselected as to sex or age. No animal which had any obvious evidence of infection or disease was accepted for a control determination. *Group II. Animals receiving a distemper vaccine.* The day of admission, after withdrawal of a control sample, 23 of these same animals were injected subcutaneously with 5 ml of canine Distemper Vaccine (Virogen).‡ This product is prepared from virulent canine distemper virus (20% suspension) and selected strains of the important secondary invaders—*Brucella bronchisepticus, Streptococcus* (pyogenic), and *Salmonella typhimurium*. It is formalin-killed, and so prepared as to preserve the full immunizing value of the virus and the bacterial antigens. They were also given an oral vermifuge (Vermiplex)§ and an insecticide was externally applied (Lexone).‖ Repeat mucoprotein determinations were made 2 days later, and thereafter at weekly intervals. *Group III. Dogs with large arteriovenous fistulas and no infection.* Mucoprotein determinations were made in 11 dogs with a severe cardiovascular stress in the form of large arteriovenous fistulas. All of these dogs had received the distemper vaccine as normal animals prior to the construction of their arteriovenous shunts. In 7 of the 11 dogs in this group the mucoprotein levels had been followed until they had returned to the normal range.¶ In the other 4 animals of this group, although actual determinations were not made, a sufficient length of time was allowed to elapse** for the mucoprotein levels to return to normal before the fistulas were made. These dogs carried either 2 lower extremity fistulas (an iliac and a femoral) or a single aorta-vena cava fistula, which were made according to technics previously described from this laboratory(7-10). The physiologic alterations in the hemodynamics and the increase in adrenal gland weights of animals with arteriovenous shunts of this size have been described(8). This group of animals had no sign of infection, and all of them had repeated negative blood cultures during the period mucoproteins were being studied. *Group IV. Dogs with large arteriovenous fistulas and bacterial endocarditis.* This fourth group of animals, in which mucoprotein determinations were made, had endocarditis produced by the standard technic employed in this laboratory(7-10). Briefly described, this method for the production of endocarditis consisted of the construction of arteriovenous fistulas of a size similar to those described for the dogs of Group III. After an interval of 4 weeks or more following construction of the shunts, small numbers (0.005 cc to 0.5 cc of a 24-hour broth culture, daily for 7 days) of Lancefield Group A†† or D‡‡ beta hemolytic streptococci were injected intravenously. These dogs then carried positive blood cultures and were proved at autopsy to have bacterial endocarditis with severe val-

‡ "Virogen®"—Pitman-Moore Co., Indianapolis.

§ Vermiplex—Pitman-Moore Co., Indianapolis.

‖ "Lexone"—E. I. du Pont de Nemours and Co., Wilmington, Del.

¶ Mean value and standard error of mean for these 7 dogs was 2.1 ± 0.27.

** 7 to 18 weeks.

†† Lancefield, Group A, Type XIV.

‡‡ Lancefield, Group D, "Strain I. F." See reference No. 10 for further description.

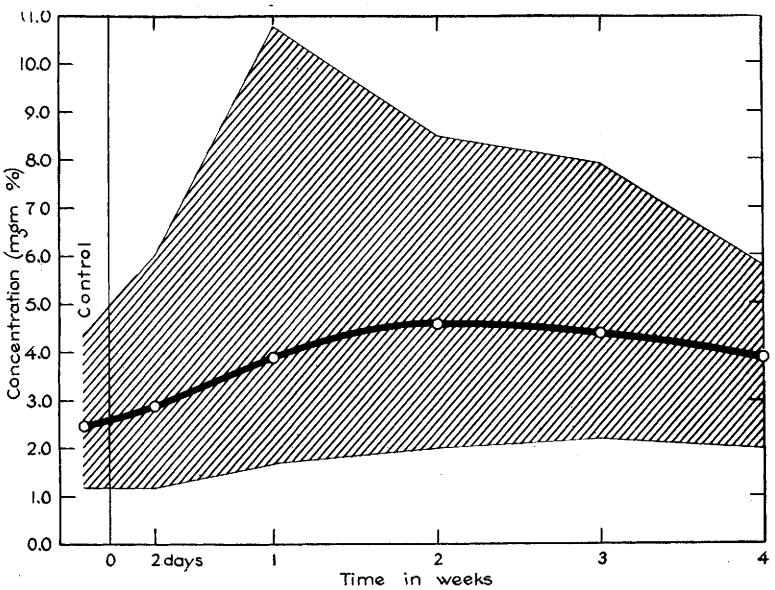

FIG. 1. Dog mucoprotein-tyrosine levels* following distemper immunization.
* (Shaded area represents the range, the heavy dark line the mean values).

vular deformities due to vegetations.

Results. Group I. Normal dogs. The mucoprotein-tyrosine value of 55 normal dogs ranged from 1.2 to 4.4 mg %. The mean value was 2.5 mg % with a standard error of the mean of ±0.11 mg %. *Group II. Animals immunized against distemper.* The sequential mucoprotein-tyrosine values for this group of dogs showed a rising trend already evident 2 days after immunization, and reaching a peak level at a time 2 weeks after the immunization. Thereafter, the values show a declining trend toward normal. In the accompanying figure (Fig. 1) the heavy line represents the mean of the values for the serum mucoprotein-tyrosine determinations in the 23 dogs followed subsequent to distemper-bacterial‡ immunization. The shaded area indicates the range of variation of these determinations. Twelve of the 23 animals were followed at weekly intervals until their mucoprotein-tyrosine values returned to normal. This occurred in the majority of animals by 7 weeks, but took as long as 18 weeks in animals which clinically developed distemper in spite of having received the vaccine. The highest (10.8 mg %) and most prolonged elevations (18 weeks) of the mucoprotein-tyrosine levels occurred in these animals. The ranges of values as shown in Fig. 1 reveal a considerable individual variation in the response to a standard vaccine. *Group III. Dogs with large arteriovenous fistulas and no infection.* The 11 dogs with large arteriovenous fistulas alone had a mean mucoprotein-tyrosine level of 3.0 mg % ±0.20 (standard error of the mean). The range in values observed varied from 1.9 to 4.0 mg %. *Group IV. Dogs with large arteriovenous fistulas and bacterial endocarditis.* The 11 dogs in this category, with large arteriovenous fistulas and a beta hemolytic streptococcic endocarditis, showed the highest mucoprotein-tyrosine levels as a group. The range for these dogs was 3.1 to 9.8 mg % with a mean of 6.0 mg % ± 0.63 (standard error of the mean).

Discussion. Statistical analyses of the results in the 4 groups of dogs studied are shown in Table I. The elevation in serum mucoprotein levels at the peak of the response to the vaccine in Group II, as compared with Group I, is significant as evidenced by a P value of $<.01$. The elevation in mucoprotein-tyrosine levels occurring in dogs with the cardiovascular stress produced by large arteriovenous fistulas only (Group III) as compared to the normal controls (Group I) is also significant ($P < .05$). The higher elevation in

TABLE I. Comparative Mucoprotein-Tyrosine Values (mg %) Statistical Analysis.

Group	No. of dogs	Range	Mean and stand. error of mean
I. Normal dogs	55	1.2–4.4	2.5 ± .11
II. " " 2 wk after immunization	23	2 –8.5	4.6 ± .36
III. Arteriovenous fistula dogs without infection or endocarditis	11	1.9–4	3 ± .20
IV. Arteriovenous fistula dogs with endocarditis	11	3.1–9.8	6 ± .63

mucoprotein-tyrosine levels in the animals with arteriovenous fistulas and endocarditis (Group IV) as compared with the controls (Group I) is highly significant (P value $<.01$). These data reveal the normal dog mucoprotein-tyrosine level, heretofore unreported, to correspond rather closely to normal human mucoprotein-tyrosine levels.

The temporary rise observed in dogs given distemper vaccine indicates that the mucoproteins may be involved in the immunological response. This observation is similar to the findings of Good *et al.*(11), who noted that a rise occurred in hyperimmunization in rabbits and rats. However, no evidence has been presented in these studies to indicate that the elevations of the serum mucoprotein levels observed in the dogs in response to distemper vaccine represents a specific immune response. This reaction of the mucoproteins may represent the non-specific response to the injection of the toxins of killed bacteria. It would be of interest to see if these observations can be clinically corroborated following routine immunization procedures in humans.

The stress of large arteriovenous fistula loads produces a small, but apparently significant increase in the dog's mucoprotein-tyrosine level. It should be noted that the stress of large arteriovenous fistulas has been shown to produce an increase in adrenal gland weight(8-10). Good and coworkers(11), in a brief report on investigations on the effect of various forms of stress on mucoproteins found an elevation to occur with chronic chilling, hyperimmunization and adrenalin injections; whereas the Arthus Phenomenon, Schwartzman Phenomenon, soft tissue destruction by cautery, adrenalectomy, and mock operation had no effect. The evidence that the serum mucoproteins reflect a stress response is, therefore, inconclusive. The highest levels of mucoproteins were observed in dogs with large arteriovenous fistulas and endocarditis, substantiating clinical observations on the mucoprotein elevation accompanying infections. The exact nature of the role of the serum mucoproteins and their ultimate significance in pathologic physiology remains to be elucidated.

Summary. 1. The normal serum mucoprotein-tyrosine level in 55 dogs has been determined. 2. The mean value for normal dogs was found to be 2.5 mg % ± 0.11, which value corresponds closely to that for normal humans. 3. Distemper vaccine produced a temporary, but significant, elevation in the mucoprotein-tyrosine levels in dogs. 4. The peak response of the serum mucoprotein-tyrosine levels to the distemper vaccine was reached in 2 weeks; and had returned to normal in the majority of animals by 7 weeks. 5. The physiologic stress of large arteriovenous fistula loads in dogs produced a statistically significant elevation in the mucoprotein-tyrosine levels. 6. The mucoprotein-tyrosine levels were found to be elevated significantly and to a much greater degree in dogs with large arteriovenous fistulas and bacterial endocarditis produced by beta hemolytic streptococci.

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Neutrality Regulation Mechanisms in Rats Receiving Sulfonic Ion Exchange Resin. (19435)

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Previous studies of the *in vivo* action of cation exchange resins(1,2) have shown that one of their important effects, when fed in either the H– or NH_4– form, is to increase urinary acid excretion (*i.e.*, $BH_2PO_4 + NH_3$). However, if the resin fed contains about 2.4 meq of sodium or potassium per g the urinary acid output remains the same as that of control animals receiving the same diet, without resin. From this it may be reasoned that the net fecal alkali loss which occurs as a result of ingesting the resin, on the specific diet used, must also be 2.4 meq per g. This assumes, of course, that there has been no net change in the alkali reserve of the animals during the experimental period. It constitutes one approach to the problem of determining the total *in vivo* cation uptake of the resin.

Our understanding of the effects of cation exchange resins on neutrality regulation mechanisms is largely based on studies of human subjects. The work of Irwin *et al.*(3) indicates that the ingestion of H-form resins results in a compensated metabolic acidosis. The fact that either H– or NH_4– form resins may cause acidosis is well known, and for the intended purpose, this is to some extent desirable(4). Rats receiving 10% of either carboxylic or sulfonic resin in their diets grow nearly as well as control animals on the same (resin-free) food(5,6), which indicates that they are quite able to deal with this situation over the long term. How they accomplish this is not exactly known, nor has the acidifying action of the resins been sufficiently studied quantitatively. The purpose of the work reported herein has been to study neutrality regulation during a period of resin feeding, and during a subsequent "recovery" period, in order to clarify some of these points more adequately.

Experimental procedures. Twelve female rats served as subjects for these experiments. They were kept in pairs in metabolism cages, with provision for the collection and separation of urine and feces. The former was collected in paraffin-lined beakers over thymol. Deionized water was available at all times. Regular colony diet* was provided *ad lib.* for 5 days before the test procedures began, and during the 5-day recovery period, except that no food was available for the last 16 hours of these periods. The resin period,† which intervened, was also of 5 days' duration; the animals were not fasted at its conclusion, but were immediately transferred to the colony diet. Food consumption records were kept for the resin and recovery periods, and all of the analytical data were reduced to terms of meq per 90 g of colony (or 100 g of resin-containing) diet consumed. For purposes of

* For composition, see reference (7), footnote 4. Analysis indicated that 90 g of this diet contained the following, as meq: Na, 27.4; K, 20.8; Ca, 29; Mg, 11; PO_4, 52; and Cl, 29.

† Diets of pairs 2-6 then contained 10% resin. Ammonium-form (4.6 meq NH_3 per g) and potassium-form (4.1 meq per g) were mixed to give potassium contents described in the Table.