other hand, the average urinary estrogen levels of chronic poliomyelitis patients were found to be higher than those observed in normal individuals. The conflicting and confusing results prompted Aycock to conclude that "endocrinopathy sought as a basis of autarceologic susceptibility does not lie in a simple deficiency in elaboration of estrogenic substance, but rather in some discrepancy in its economy." A similar conflict has been noted in the case of the pregnant woman(3) and it has been suggested that fluctuations in resistance during gestation may depend upon variations in relative dominance of the manifold endocrine constituents rather than upon quantitative changes of any individual hormone in the system(20). However, the argument in favor of a protective function of chorionic gonadotropin in early pregnancy would appear to stand upon less conflicting evidence contributed by other investigators(17,21).

More recently, the influence of various endocrine substances was studied anew by Anderson and Bolin(22) in mice orally infected with the MM strain of murine poliomyelitis virus. While stilbestrol or progesterone treatment afforded a high order of protection, mice treated with t-propionate (presumably testosterone-propionate) were only partially protected, and those receiving desoxycorticosterone-acetate were as susceptible as the untreated animals. Again, it was suggested that the protective effects might have been due to alterations in the permeability of mucous surfaces preventing entry of the virus. In our work, however, there was no significant difference in the results which could be ascribed to the route of inoculation, whether oral or intravenous. Thus, our own observations serve to emphasize once more the possibility that the state of pregnancy may be associated with a systemic increase in susceptibility to poliomyelitis, irrespective of any changes that may exist at the portal of entry.

*Conclusions.* Pregnant mice inoculated with the Col. SK strain of murine poliomyelitis virus by the oral route showed a definite increase in susceptibility to infection as compared with non-pregnant female control animals. Beginning with the 4th day of pregnancy, mortality increased progressively and reached, with the last 4 days of gestation, a rate almost twice that of the control group. Immediately after parturition there was a rapid return of resistance to the non-pregnant level. The mechanism responsible for the greater susceptibility of the pregnant mouse does not seem to depend wholly upon alterations in the permeability of the intestinal mucosa during gestation, since a smaller group of mice infected by the intravenous route exhibited a similar increase in susceptibility.

20. Davis, W. D., and Silverstein, C. M., *U. S. Naval Med. Bull.*, 1947, v47, 910.

21. Jungeblut, C. W., Meyer, K., and Engle, E. T., *J. Immunol.*, 1934, v27, 43.

22. Anderson, J. A., and Bolin, V., *Endocrinol.*, 1946, v39, 67.

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## Further Observations on the "Animal Protein Factor." (17731)

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A previous publication(1) from this laboratory has shown that an "animal protein factor" supplement (Fraction 1) made from cultures of *Streptomyces aureofaciens* produced a growth response in chicks which was greater than the maximum growth response obtainable with vitamin $B_{12}$. Distillers' solubles, fish meal, fish solubles and dried brewers' yeast did not produce additional growth

1. Stokstad, E. L. R., Jukes, T. H., Pierce, J. V., Page, A. C., Jr., and Franklin, A. L., *J. Biol. Chem.*, 1949, v180, 647.

TABLE I.
Composition of Diets.

| Ingredient | Diets 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Soybean meal, expeller process | 30 | 70 | — | — | — | — |
| Fish meal | — | — | 25 | — | — | — |
| Cottonseed meal | — | — | — | 35 | — | — |
| Peanut meal | — | — | — | — | 35 | — |
| Sodium proteinate | — | — | — | — | — | 25 |
| Yellow corn meal | 63 | 23 | 69 | 57.4 | 57.4 | — |
| Cerelose | — | — | — | — | — | 59.7 |
| Bone ash | 1.0 | 1.0 | — | 1.0 | 1.0 | 2.5 |
| Calcium carbonate | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | — |
| " gluconate | — | — | — | — | — | 5.0 |
| Salt mixture* | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 | 1.4 |
| Sodium chloride | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.6 |
| Vit. mixture in glucose† | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Corn oil + vit. A, D, and E‡ | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 3.0 |
| Methionine (DL–) | 0.3 | 0.3 | 0.3 | 0.4 | 0.4 | 0.3 |
| Choline chloride | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Lysine (DL–) hydrochloride | — | — | — | 0.5 | 0.5 | — |
| Glycine | — | — | — | — | — | 1.0 |
| Cystine | — | — | — | — | — | 0.3 |

* The salt mixture had the following composition: $K_2HPO_4$ 300, $KH_2PO_4$ 225, $MgSO_4$ 125, $MnSO_4$ (anhydrous) 20, ferric citrate 25, $CuSO_4 \cdot 5H_2O$ 1.0, KI 0.3, zinc acetate 0.7, $Al_2SO_4 \cdot 18H_2O$ 0.8, cobalt acetate 0.2, nickel carbonate 0.1 (Total 715).

† The vit. mixture contributed the following per kg of diet: Thiamine HCl 10 mg, riboflavin 10 mg, niacinamide 50 mg, pyridoxine 10 mg, calcium pantothenate 50 mg, pteroylglutamic acid 2 mg, biotin 0.2 mg, 1-acetoxy-2-methyl-4-napthyl sodium phosphate (vit. K derivative) 5 mg.

‡ Vit. A acetate 1,500 U.S.P. units, vit. $D_3$ 200 A.O.A.C. units, mixed tocopherols 34 mg dissolved in 1.0 g corn oil.

above that obtained with vitamin $B_{12}$. Cunha *et al.*(2) noted that the "animal protein factor" supplement produced a marked response in pigs on a corn-peanut meal basal diet while a vitamin $B_{12}$ concentrate had little effect. McGinnis and co-workers reported(3) that on a 56% soybean diet, a liver extract containing vitamin $B_{12}$ produced no growth response in young turkeys while the "animal protein factor" supplement gave a marked growth increase. The growth-promoting effects of various supplements upon chicks receiving a basal diet containing 70% of soybean meal(1) and adequately supplied with vitamin $B_{12}$ were studied in the present investigation. It was found that either crystalline aureomycin hydrochloride or a culture of *S. aureofaciens* in which the aureomycin had been inactivated had growth-promoting effects.

*Methods.* Depleted chicks with a low reserve of the animal protein factor were obtained by hatching eggs from hens on Diet 1(1). The chicks (Barred Rock x New Hampshire) were placed immediately after hatching on the experimental diets. Two types of aureomycin fermentation products were used.* Fraction 1 was a dry mixture of the mycelium plus a variable amount of filter-aid. A concentrate of the whole fermented mash from which water had been removed by evaporation was also used. Vitamin $B_{12}$ was supplied either in the crystalline form or as a mixture of vit. $B_{12}$ and $B_{12b}$ containing more than 10 $\mu$g of the mixed vitamins per mg of organic solids. The alkaline extract of fraction 1 was made by heating 1 kg of this fraction with an excess of 0.5 N sodium hydroxide, neutralizing and concentrating.

2. Cunha, T. J., Burnside, J. E., Buschman, D. M., Glasscock, R. S., Pearson, A. M., and Shealy, A. L., *Arch. Biochem.*, 1949, v23, 324.

3. McGinnis, J., Stephenson, E. L., Lavadie, B. T., Carver, J. S., Garibaldi, J. A., Ijichi, K., Snell, N. S., and Lewis, J. C., Paper presented at the Am. Chem. Soc. Meeting, Atlantic City, N. J., Sept. 1949.

* The aureomycin fermented mash and fraction 1 were kindly supplied by Dr. S. H. Babcock and Dr. A. S. Phelps, Chemical Production Section, Lederle Laboratories.

TABLE II.
Response Produced by Vit. $B_{12}$ and Other Supplements on Different Types of Basal Diets.

| Exp. No. | Type of basal ration and supplement per kg of diet | | Wt and survivors ( ) at 25 days, g |
|---|---|---|---|
| I. | Diet 2 (70% soybean meal) | 30 μg $B_{12}$ | 281 (18)* |
| | | 30 " " + 15 g fraction No. 1 | 360 (12) |
| | | 30 " " + 30 ml conc. mash No. 2 | 368 (10) |
| | Diet 3 (25% fish meal) | 30 " " | 295 (10) |
| | | 30 " " + 15 g fraction No. 1 | 346 (11) |
| II. | Diet 1 (30% soybean meal) | $B_{12}$ injection† | 247 (12) |
| | | " " + 50 g dried whey | 242 (11) |
| | | " " + 4 g fraction No. 1 | 286 (12) |
| | Diet 2 (70% soybean meal) | " " | 260 (11) |
| | | " " + 8 g fraction No. 1 | 292 (11) |
| | Diet 4 (35% cottonseed meal) | None | 182 (12) |
| | | $B_{12}$ injection | 185 (11) |
| | | " " + 4 g fraction No. 1 | 216 (11) |
| | Diet 5 (35% peanut meal) | None | 239 (11) |
| | | $B_{12}$ injection | 240 (12) |
| | | " " + 4 g fraction No. 1 | 288 (10) |

* 24 chicks used initially in this group, 12 used in all others.
† 4 μg intramuscularly per week.

This extract showed no aureomycin activity when assayed† with *Staphylococcus aureus* by the method of Dornbush and Pelcak (4).

*Results. Amount of vit. $B_{12}$ needed for maximum growth:* It was necessary to supply sufficient $B_{12}$ so that any additional amounts of $B_{12}$ in the supplements to be tested did not produce an additional growth response. A number of experiments were made to determine the requirements for vitamin $B_{12}$. When given in the diet the requirement varied from 15 to 30 μg per kg of diet. Intramuscular injection of vitamin $B_{12}$ was also used. About 0.4 μg injected once weekly has been found to meet the vitamin $B_{12}$ requirement of chicks (1). In the assays for the unknown growth factor, 30 μg of vitamin $B_{12}$ were added per kilo of diet or injections of 4 μg were given on the 1st, 7th and 15th days.

*Response obtained on different types of diet.* Basal diets containing various protein concentrates were used and their composition is shown in Table I. In diets 4 and 5, containing cottonseed meal and peanut meal, lysine was included to make the diets more adequate with respect to amino acids and thus to eliminate the possibllity of the production of growth responses by any lysine which might have been present in the supplements being tested. The effects of vitamin $B_{12}$ with or without aureomycin fermentation fractions were tested with chicks and the results are shown in Table II. These fermentation fractions produced responses in chicks on diets containing either 30 or 70% soybean meal. It was found that vitamin $B_{12}$ produced no response when added to the diets containing fish meal, cottonseed meal and peanut meal. However, the aureomycin fractions produced a growth response when added to any of the diets and the percentage increase in growth over that obtained with the basal diets was approximately the same for all types of diets. The results with fish meal were of interest in that they indicated that fish meal did not furnish effective amounts of the unknown growth factor. Dried whey produced no growth response when added to diet 1 in the presence of vitamin $B_{12}$.

*Response to Crystalline Aureomycin.* Chemical fractionation of the aureomycin mash revealed some similarities between the distribution of aureomycin and that of the growth factor. Aureomycin hydrochloride which had been repeatedly recrystallized‡ was

† Assays for aureomycin were made through the courtesy of Miss Frances L. Clapp and Miss Frances Bingham, Lederle Laboratories.

4. Dornbush, A. C., and Pelcak, E. J., *Ann. N. Y. Acad. of Sci.*, 1948, v51, 218.

‡ This sample of specially purified aureomycin was supplied through the courtesy of Dr. R. Winterbottom, Lederle Laboratories.

TABLE III.
Growth Stimulation Produced in Chicks by Various Supplements in Presence of Vitamin $B_{12}$.

| Supplement per kg of diet 2 | | Avg wt and No. of survivors ( ) Experiment No. III* 21 days | IV* 25 days | V* 21 days | VI† 25 days | VII* 21 days |
|---|---|---|---|---|---|---|
| None | | 248 (12) | 249 (11) | 207 (11) | 246 ( 5) | 221 (11) |
| " | | | 268 (11) | | 269 ( 7) | 223 (12) |
| Concentrated aureomycin mash | 2.5 ml | | | 247 (11) | | |
| " " " | 10 " | | | 249 (11) | | |
| " " " | 30 " | 288 (12) | 298 (11) | | | 273 (12) |
| " " " | 30 " | | | | | 294 (11) |
| Aureomycin HCl | 25 mg | | | 235 (11) | | |
| " " | 60 " | 272 (11) | | | | |
| " " | 100 " | | 309 (11) | 242 (11) | | 270 (11) |
| Fraction 1 | 5 g | | | | 337 (12) | |
| " " | 15 " | | | | 343 (12) | |
| Alkaline extract of 10 g, Fraction 1 | | | | | 329 (12) | |
| " " " 30 " " " | | | | | 342 (12) | |
| Streptomycin, $CaCl_2$ complex | 100 mg | | | | | 255 (12) |
| " " " | 300 " | | | | | 266 (12) |
| Succinylsulfathiazole | 10 g | | | 228 (10) | | |
| 3-nitro-4-hydroxy-phenyl-arsonic acid | 50 mg | | | | | 265 (12) |
| " " " " " " " | 100 " | | | | | 241 (12) |

* 4 $\mu$g vit. $B_{12}$ injected weekly.
† 30 " " " added per kg of diet.

found to produce a growth response. Some fractions were obtained from fermented mash which were almost devoid of aureomycin but which still retained growth activity. Furthermore, an extract prepared by heating fraction with 0.5 N NaOH gave a growth response in chicks although all the aureomycin had been destroyed by the treatment. Aureomycin fermented whole mash was also heated with sodium hydroxide without destroying the growth-promoting activity, although all the aureomycin had been inactivated. On the basis of a large number of experiments crystalline aureomycin did not appear to give uniformly as great a response as the aureomycin whole mash. It seems unlikely that all the growth-promoting activity of the fermented mash can be accounted for on the basis of its aureomycin content.

Since the addition of succinylsulfathiazole and streptomycin to a purified diet have been reported to produce a growth-promoting effect(5), these compounds were tested on diet 2. The results which are presented in Table III, show that succinylsulfathiazole produced a response which was not as large as that produced by aureomycin hydrochloride or aureomycin mash. A level of 100 mg of streptomycin gave a partial increase while significant responses with aureomycin were obtained with levels as low as 25 mg per kg of diet. A response was obtained with 300 mg of streptomycin. This is in agreement with the results of Moore and co-workers(5) who found that 500,000 units of streptomycin, (*ca* 500 mg) per kg of a purified diet gave a better response than 200,000 units, (*ca* 200 mg).

Morehouse and Mayfield(6) and Morehouse(7) have described the growth stimulation produced in chickens and turkeys by 3-nitro-4-hydroxyphenylarsonic acid. Bird and co-workers(8,9) have also presented evidence on the growth-promoting activity of various phenylarsonic acid derivatives with

5. Moore, P. R., Evenson, A., Luckey, T. D., McCoy, E., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1946, v165, 437.

6. Morehouse, N. F., and Mayfield, O. J., *J. Parasitol.*, 1946, v32, 20.

7. Morehouse, N. F., *Poultry Science*, 1949, v28, 375.

8. Bird, H. R., Groschke, A. C., and Rubin, M., *Fed. Proc.*, 1948, v7, 283.

9. Bird, H. R., Groschke, A. C., and Rubin, M., *J. Nutrition*, 1949, v37, 215.

TABLE IV.
Effect of Various Supplements on "Sodium-Proteinate" Diet—Basal Diet No. 6. (12 chicks per group).

| Vit. $B_{12}$ per kg diet, $\mu$g | Supplement per kg diet | Wt and survivors ( ) 25 days, g |
|---|---|---|
| None | None | 173 ( 9) |
| 50 | " | 229 (11) |
| 50 | Brewer's yeast 75 g | 250 (12) |
| 50 | Fish meal 50 g | 234 (12) |
| 50 | Aureomycin fermented mash 30 ml | 330 (12) |

chicks. In the experiments summarized in Table III, growth responses were obtained with 3-nitro-4-hydroxyphenylarsonic acid although no level of this compound which was used produced gains as large as that obtainable with aureomycin mash.

*Growth response produced on soybean protein diet.* Carlson *et al.*(10) have reported that yeast contained an unidentified growth factor, as evidenced by the response produced on a diet containing "alpha-protein" and supplemented with 11.6 $\mu$g of vitamin $B_{12}$ per kg of diet. With Diet No. 6, similar to that used by Carlson(10) except that "sodium proteinate"§ was used in place of "alpha-protein," we obtained only a slight response with yeast and no response with fish meal when 50 $\mu$g of vitamin $B_{12}$ were added per kg of basal ration. These results, which are shown in Table IV, also show that aureomycin fermented mash produced a gain of 53% above that produced on the basal diet supplemented with $B_{12}$. Thus, it is apparent that the nature of the growth response produced by aureomycin fermentation products is distinct from that described by Carlson and co-workers(10). The ineffectiveness of whey (Table II) appears to distinguish the growth response described here from that produced by the "whey factor" described by Hill(11).

*Discussion.* The growth stimulation which was observed in chicks on aureomycin fermentation materials can be attributed, at least in part, to aureomycin. Unpublished experiments(12) indicate that aureomycin produces growth responses in pigs and turkeys under conditions(2,3) in which these species were reported to respond to fraction 1.

The possibility that the growth produced by aureomycin is related to its antibiotic activity is raised by our experiments. The fact that growth responses have been obtained by such widely differing types of compounds as succinylsulfathiazole, streptomycin and substituted phenylarsonic acids supports this view. Harned *et al.*(13) in a single experiment with two groups of 6 chicks reported that the addition of aureomycin increased the growth rate and attributed the effect to the elimination of some infection. No infections were noted in the present investigation.

*Summary.* 1. Fermentation products of *Streptomyces aureofaciens* were found to promote growth in depleted chicks on various diets which were adequately supplied with vitamin $B_{12}$.

2. Growth responses in chicks on a corn-soybean diet were also produced by crystalline aureomycin hydrochloride and by cultures in which the aureomycin, as measured by antibiotic potency, was destroyed by alkaline hydrolysis.

3. Responses were also obtained with succinylsulfathiazole, streptomycin and 3-nitro-4-hydroxyphenylarsonic acid, but these substances appeared less potent than aureomycin. No responses were obtained with dried whey or dried brewer's yeast.

*Addendum.* Following the submission of this manuscript, Dr. Paul Gyorgy kindly showed us a manuscript (Gyorgy, P., Stokes, J., Jr., Smith, W. H., and Goldblatt, H., *Transactions of Conference on Biological*

10. Carlson, C. W., Miller, R. F., Peeler, H. T., Norris, L. C., and Heuser, G. F., *Poultry Science*, 1949, v28, 750.

§ A purified protein prepared from soybean meal and obtained through the courtesy of Archer-Daniels-Midland Company.

11. Hill, F. W., *Poultry Science*, 1948, v27, 536.

12. Stokstad, E. L. R., Jukes, T. H., Taylor, R. R., Cunha, T. J., Edwards, H. M., and Meadows, G. B., *Arch. Biochem.*, 1950, in press.

13. Harned, B. K., Cunningham, R. W., Clark, M. C., Cosgrove, R., Hine, C. R., McCauley, W. J., Stokey, E., Vessey, R. E., Yuda, N. N., and Subba-Row, Y., *Ann. N. Y. Acad. Sci.*, 1949, v51, 182.

*Antioxidants,* sponsored by the Macy Foundation, November, 1949) which described a beneficial effect exerted by aureomycin on growth and the prevention of dietary hepatic necrosis in rats. The assumption was made that aureomycin might act through the suppression of the intestinal flora.

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## An Improved Vertical Cylinder Oxygenator.* (17732)

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Prolonged interruption of blood flow to the heart and lungs followed by survival of the animals has been accomplished(1). The blood was oxygenated extracorporeally by blowing oxygen over a blood film upon the inner surface of a revolving vertical metal cylinder of 0.23 sq. meter surface area. A larger oxygenator of this type was described in detail(2). Recently, a still larger vertical cylinder, 79 cm in height, and 30 cm in internal diameter, has been constructed which differs from those previously described only in having a larger surface area, 0.71 sq. meter. It was thought that if the blood flow on the smooth inner surface of the cylinder could be made turbulent, the capacity of the apparatus to introduce oxygen could be increased. The basic apparatus was therefore modified by closely adapting a 16-mesh, 26-gauge wire screen to the cylinder's entire inner surface.

Data showing the relative oxygenating efficiency with and without the screen are shown in Table I. These values for oxygen introduced by a single passage of blood over the oxygenating surface were chosen from a number of determinations for the similarity of their initial venous oxygen saturations.

The maximum amount of oxygen which

TABLE I.
Oxygenating Capacity—Screened and Smooth Surfaces.

| Observation No. | Type of surface | $O_2$ content vol. % | | $O_2$ capacity Vol. % | % saturation | | Bld flow ml/min. | cc $O_2$ added per 100 ml blood | Total cc $O_2$ added per min. |
|---|---|---|---|---|---|---|---|---|---|
| | | Venous | Arterial | | Venous | Arterial | | | |
| 1 | Smooth | 8.9 | 14.8 | 21.9 | 40.6 | 67.4 | 1080 | 5.8 | 63.1 |
| 2 | Screen | 8.7 | 21.9 | 21.8 | 40.1 | 100.9 | 856 | 13.2 | 113.3 |
| 3 | Smooth | 8.9 | 15.5 | 21.9 | 40.6 | 70.8 | 1080 | 6.6 | 71.3 |
| 4 | Screen | 8.7 | 21.4 | 21.8 | 40.1 | 98.5 | 1360 | 12.7 | 172.2 |
| 5 | Smooth | 9.2 | 16.5 | 18.9 | 48.2 | 87.0 | 510 | 7.3 | 37.0 |
| 6 | Screen | 8.2 | 14.8 | 17.2 | 47.7 | 86.2 | 8200 | 6.6 | 542.8 |
| 7 | Smooth | 10.8 | 15.1 | 18.4 | 58.7 | 82.1 | 1125 | 4.3 | 48.4 |
| 8 | Screen | 9.8 | 16.0 | 18.1 | 54.1 | 88.5 | 2000 | 6.2 | 124.0 |
| 9 | Smooth | 10.8 | 15.6 | 18.4 | 58.7 | 84.8 | 1000 | 4.8 | 48.0 |
| 10 | Screen | 9.8 | 14.9 | 18.1 | 54.1 | 82.3 | 7160 | 5.1 | 365.0 |
| 11 | Smooth | 11.1 | 14.7 | 18.4 | 60.3 | 79.8 | 1210 | 3.6 | 43.5 |
| 12 | Screen | 11.2 | 14.2 | 17.2 | 65.2 | 82.5 | 7160 | 3.0 | 215.0 |
| 13 | Smooth | 11.1 | 16.0 | 18.4 | 60.3 | 86.9 | 1000 | 4.9 | 49.0 |
| 14 | Screen | 11.2 | 14.7 | 17.2 | 65.2 | 85.5 | 7160 | 3.5 | 251.0 |

Single passage over smooth and screen oxygenating surfaces at comparable initial oxygen saturation levels. Venous blood is blood before passage and arterial after passage.

* Aided by a grant from the National Heart Institute of the United States Public Health Service.

† Fellow in Research Surgery.

1. Gibbon, J. H., Jr., *Surg., Gynec. and Obst.,* 1939, v69, 602.

2. Gibbon, J. H., Jr., and Kraul, Charles W., *J. of Lab. and Clin. Med.,* 1941, v26, 1803.