TABLE I. Comparative Effects of Various Substances on Infant Rats. Injections Begun at One Day of Age.

Substance	Daily dose	Days treated	Total dose	Retardation of growth	Hastening of tooth eruption and eye opening
Cortisone	.1 mg	5	.5 mg	4+	4+
Aqueous adrenal extr.(1)	.2 ml	10	2 ml	0	3+
Desoxycorticosterone	.25 mg	14	3.5 mg	0	3+
Corticosterone	.2 "	8	1.6 "	0	2+
ACTH	.75 "	8	6 "	2+	±
Pregnenolone	5. "	13	65 "	0	0

stances. It also reflects the fact that the infant rat can withstand much larger doses of DCA than cortisone. This is the reverse of what occurs in man where relatively small doses of DCA are toxic as compared with the dose of cortisone. The dose of DCA that can be administered to man is limited by virtue of its marked effect on electrolyte and water balance. Apparently, this sign of toxicity is not manifested in the infant rat. The present experiments do not shed light on the mechanism of the precocious changes. Both the adrenal hormones affecting carbohydrate metabolism and those affecting electrolyte metabolism produce these changes. It does not appear to be due to a non-specific effect of the steroids because pregnenolone which structurally resembles the other substances is ineffective.

Summary. 1. ACTH, cortisone, corticosterone, desoxycorticosterone and pregnenolone were injected into newborn rats in varying dosage. Cortisone was found to be the most toxic with a total of 0.6 mg in the first few days of life being a lethal dose, as contrasted with the lack of toxicity of 0.25 mg of desoxycorticosterone given daily for 14 days. Cortisone was the most potent growth inhibitor and also the most potent stimulus for eruption of teeth, opening of eyelids and development of the gingivae. ACTH, although employed in larger doses than cortisone had a lesser effect on growth inhibition and little or no effect on development. Larger doses of ACTH are yet to be used. Corticosterone in the dose employed did not affect growth but did stimulate development. Pregnenolone in huge doses had no effect on either growth or development of teeth and eyelids. 2. Cortisone in a total dose of 0.5 mg (sub-lethal) given to newborn rats in the first week of life resulted in long-term damage to the animal as evidenced by failure to regain normal body weight 3 months after cessation of injections. The younger the animal at the time of the first injection, the more marked was the growth failure.

Received April 26, 1951. P.S.E.B.M., 1951, v77.

Content of Radioactive Vitamin B_{12} in the Feces of Rats Fed Co^{60} and Aureomycin.* (18729)

ROBERT L. DAVIS AND BACON F. CHOW.

From the Department of Biochemistry, School of Hygiene and Public Health, The Johns Hopkins University.

The importance of trace elements such as cobalt for optimal growth and normal health was long recognized by McCollum(1). Gall (2) demonstrated that the oral administration

* This work was supported by grant-in-aid from Sharp and Dohme, Merck and Co., and Upjohn and Co.

1. McCollum, E. V., Orent-Keiles, E., and Day, H. G., *The Newer Knowledge of Nutrition,* New York, 1944, The Macmillan Co.

of "cobalt" resulted in stimulation of certain bacterial organisms in the rumen of sheep. In a similar experiment Abelson and Darby (3) fed radioactive cobalt (Co^{56}) and isolated from feces a fraction soluble in n-butanol and containing radioactive Co^{56}. This fraction was found, also, to possess the microbiological activity of vit. B_{12} as measured by *L. leichmannii*. These reports, therefore, indicate that at least a part of cobalt in the diet is utilized for the synthesis of vit. B_{12} molecule, presumably by the organisms in the intestinal flora. Stokstad(4) and his associates found that growth responses of chicks on a corn-soybean diet adequately supplied with vit. B_{12} were stimulated as a result of supplementation with crystalline aureomycin and less effectively by other bactericidal agents such as succinyl-sulfathiazole, streptomycin, and 3-nitro-4-hydroxy-phenyl arsonic acid. Similar results from experiments using swine were reported by Edwards *et al.*(5) and Jukes *et al.*(6). On the other hand, studies by Colby *et al.*(7) demonstrated that feeding aureomycin to lambs produced harmful effects, such as decrease in feed consumption and loss in body weight. They attributed their observations to the inhibition of certain strains of bacteria in the intestinal flora by aureomycin.

The above data demonstrate that the addition of aureomycin in excessive amounts may result in deleterious effects, whereas supplementation in appropriate amounts may stimulate growth response, possibly through alteration of the flora so as to result in an increase in the synthesis of certain vitamins utilizable by the host. Direct experimental evidence for the latter effect, however, is still lacking. Hence, it would be of interest to demonstrate, if possible, an increase in the synthesis of vitamins by comparing the contents of a certain selected vitamin in the fecal matter of rats fed a diet with or without supplementation with aureomycin. Since the vit. B_{12} molecule contains an atom of cobalt which can be isotopically tagged, the synthesis of the vitamin by the micro-organisms in the intestines can be readily followed. This communication reports the results of such a study.

Experimental procedure. The composition of the control soybean diet is: 62% soybean meal, 29.5% sucrose, 4% salts mixture IV, and 4.5% corn oil. The vitamin supplement for each kg diet consists of 2.0 mg thiamine, 3.0 mg riboflavin, 2.5 mg pyridoxine, 20.0 mg calcium pantothenate, 50 mg niacin, 100 mg inositol, 0.1 mg biotin, 250 mg p-aminobenzoic acid, 0.2 mg folic acid and 1 g choline HCl, 210 mg percomorph oil, 23 mg vit. E, 2.1 mg vit. K, 12,600 units vit. A, and 1,785 units vit. D. Approximately year-old male and female rats were used for the experiment. They were raised from the time of birth on the control soybean diet deficient of vit. B_{12}. At the beginning of the experiments the males weighed approximately 380 g and the females 300 g. They were kept in individual metabolism cages and were acclimated to the control diet, with or without aureomycin supplement for a period of 7 days. Their dietary intake was limited to 12 g per rat per day during the entire period of study. On the eighth day, one ml of $CoCl_2$ solution containing 0.2 to 0.4 μg of radioactive cobalt $\simeq$ 20,000 to 40,000 c.p.m. was measured into every portion of the daily dietary allowance of rats in both the control and aureomycin groups (Table I). Urine specimens were discarded. Feces were collected every 48 hours. They were weighed and homogenized with about 80 ml of cold distilled water, and the total volume was recorded. After centrifugation in the cold, a 10 ml aliquot of the clear homogenate was removed, and an equal volume of saturated ammonium sulfate was added to it. The solution was extracted 4 times with equal volumes of normal butanol.

2. Gall, L. S., Smith, S. E. Becker, D. E., Stark, C. N., and Loosi, J. K., *Science*, 1949, v109, 468.

3. Abelson, Philip H., and Darby, Hugh H, *Science*, 1949, v110, 566.

4. Stokstad, E. L. R., and Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 523.

5. Edwards, H. M., Cunha, T. J., Meadows, G. W., Sewell, R. F., and Shawver, C. B., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 445.

6. Jukes, T. H., Stokstad, E. L. R., Taylor, R. R., Cunha, T. J., Edwards, H. M., and Meadows, G. W., *Arch. Biochem.*, 1950, v26, 324.

7. Colby, R. W., Rau, F. A., and Dunn, R. C., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 234.

TABLE I. Radioactivity* of the Butanol Extracts of Feces of Rats on Various Test Diets.

Group	No. of rats	% aureomycin in diets	μg† Co^{60} added/day	Assay period (48 hr) 1st	2nd	3rd	4th	5th
W	6	.0	.2	1.3	1.5	1.5	1.7	1.6
X	6	.04	.2	3.1	6.1	5.1	5.1	5.7
Y	3	.1	.2	4.4	5.3	6.0	7.0	5.6
U	3	.1	.4	8.2	10.2	9.5	10.8	8.8
Z	3	.0	.4	3.3	—	—	—	—

* Avg of group in c.p.m. × 10^{-3}.
† One μg ≏ 100,000 c.p.m.

The butanol extracts were pooled and centrifuged to remove particulate matter of the aqueous phase that might have been carried over mechanically. A small aliquot (0.5 ml) of supernatant was saved for microbiological assay which was not affected by the amount of butanol present after the necessary dilution of some 100-fold. The remaining portion of butanol extract (approximately 80 ml) was boiled off until the volume was reduced to approximately 2-3 ml, the concentrate was transferred quantitatively to a counting pan. The contents were dried, and the radioactivity of the solid was measured with Geiger-Müller instrument.

The data in Table I indicate that the amount of radioactivity of the cobalt compound, soluble in the butanol extract of fecal matter in any single group of rats, was essentially constant over the 5 periods of observation. It can be noted that the addition of aureomycin to the diet brought about a several-fold increase in the radioactivity (compare Groups W and X). It appears that supplementation with 0.04% aureomycin produced a maximum effect since a higher level of the antibiotic did not bring about systematically any added increase in radioactivity (compare Groups X and Y). However, when the daily cobalt supplement was raised to 0.4 μg per rat the radioactivity was increased (compare Groups U and Y). Animals in Group Z received the same amount of cobalt chloride but no antibiotics. Although we have data only for the first period, they indicate that the addition of $cobalt^{60}$ may be a limiting factor in the biosynthesis of the organic Co-complex in the intestinal flora. This is in harmony with the results of Hendlin and Ruger(8) who studied, *in vitro,* the cobalt requirements of *S. griseus.*

Even though results in Table I demonstrate the increase in the radioactivity in the butanol extract following supplementation of aureomycin, this effect might be accounted for if our procedure does not quantitatively free the organic phase from the inorganic cobalt salt. To eliminate such a possibility, we have added 0.4 μg $cobalt^{60}$ ≏ 40,000 c.p.m. and 313 mμg of vit. B_{12} ≏ 3130 c.p.m. to a fecal sample from a rat previously offered the control diet free from any radioactive substance. The recovery in the butanol phase, by analyses of radioactivity, was 2,400 c.p.m. or 75% in terms of radioactive B_{12} added and by microbiological analysis was 250 mμg or 80% of vit. B_{12} added. These data, therefore, show satisfactory separation of vit. B_{12} from $CoCl_2$ under our experimental conditions. Since the cobalt-containing compound soluble in the butanol phase is essentially free from the cobalt ion, it is conceivable that cobalt might be coordinated with some organic molecules to form compounds soluble in normal butyl alcohol. Such compounds will possess radioactivity of $cobalt^{60}$ but need not have the microbiological activity of vit. B_{12}.

It is, therefore, important to correlate radioactivity with microbiological activity of the fraction soluble in butanol. The data collected in Table II demonstrate that Group W, which has the lowest count, has the least microbiological activity, whereas Group U is highest in both. The ratios of radioactivity to microbiological activity of the fecal samples from different groups of rats are essentially constant (see last column). According to this constant, there were about 2 x 10^{-3} c.p.m. per μg of vit. B_{12} found by microbiological assay. The results, therefore, suggest that the butanol phase contains vit. B_{12} or B_{12}-like substance. Additional evidence may be obtained by correlating other physical characteristics of vit. B_{12} with the radioactivity of Co^{60}. However, final proof could be

8. Hendlin, D. and Ruger, Myrle L., *Science,* 1950, v111, 541.

TABLE II. Correlation of Microbiological and Radioactivity in the Butanol Extract.

Group*	No. of rats	A = Radioactivity avg of group c.p.m. × 10^{-3} per sample	B = Microbiological activity group avg expressed in γ/sample B_{12}	A/B
W	6	2	.9	2.2
X	3	6	2.8	2.1
Y	2	5	2.0	2.5
U	3	9	3.4	2.6

* See Table I for composition of group diets.

TABLE III. Fecal Weights of Rats on Diets with or Without Aureomycin.

Group*	No. of fecal samples	Sex	Avg wt of feces in g/2-day collection
W	26	F	3.1 ± .23
X	26	F	4.2 ± .32
Y	6	M	5.3 ± .3
U	11	M	6.3 ± .4

* See Table I for composition of group diets.

reached only by actual isolation.

Another observation which is worth recording deals with the relative fecal matters collected from different groups of rats. The fecal weights are tabulated in Table III. Although all animals had approximately the same diet allowance, the feces of those in the aureomycin groups (X, Y, and U) weighed more than those in the control group W. Furthermore, this difference in the fecal weight manifested itself not only during any single period but was sustained during the entire experiment.

Discussion. The fact that the addition of antibiotics in appropriate amounts can improve the nutritional status of the host can be accounted for on the assumption that the bactericidal agent selectively destroys certain microorganisms, (a) which will otherwise compete with the host for accessory factors synthesized by another group of organisms, or (b) which produce toxins that might interfere with the utilization of nutrients. The data presented in this paper provide direct evidence that the addition of aureomycin results in an increase in the vit. B_{12} content of the fecal matter. Our experiments do not exclude the possibility that the fecal content of other accessory factors might also have been increased as a result of aureomycin feeding. Such an hypothesis could explain some of the findings of Jukes *et al.*, who demonstrated some beneficial effects to growing animals after supplementation of aureomycin in diets with adequate supply of vit. B_{12}. It is also important to point out that, in this series of experiments, only aureomycin was used, although other antibacterial agents might be expected to produce similar effects.

Summary. By the incorporation of Co^{60} into the diet, it could be shown that aureomycin causes an increase in the activity, both radioactive and microbiological, of the vit. B_{12} content of the feces. The fecal B_{12} content could be increased by increasing the amount of Co^{60} used. The experimental findings can be explained by the effect of the antibiotic on the intestinal bacterial flora.

Received April 27, 1951. P.S.E.B.M., 1951, v77.

Filtrates of Fresh Tumor Injected Prior to Transplantation of the Homologous Tumor.* (18730)

ALBERT E. CASEY, GEORGE DRYSDALE, GORDON L. ROSS, AND BRUCE A. ELROD.

From the Departments of Pathology, Birmingham Baptist Hospital and Medical College of Alabama.

Anaerobic refrigeration in the deep freeze (0-24°F) for 10 days or longer (until the cells are no longer viable) or lyophilization for a similar period of Brown-Pearce tumor tissue(1,2), Bashford Ca 63(3-5), 15091a (6-8) or Eo771(6-9) tumor tissue results in the presence of specific(5-9) substances which,

* This work was aided by grants from the American Cancer Society and the Damon Runyon Fund through the Committee on Growth of the National Research Council and by the International Cancer Research Foundation.