is, however, known to vary considerably and, moreover, the drying procedure may possibly result in an inferior serum from the standpoint of volume of growth. On the other hand, Hetherington has reported excellent results with desiccated media(7,8).

The efficacy of aqueous humor as a tissue culture nutrient suggests that the success of intra-ocular transplantation depends on the ability of the fluid to maintain cells for relatively long periods or until vascularization occurs permitting continued growth.

Summary. (1) Pure aqueous humor from beef eyes will support survival but only limited growth of chick fibroblasts. (2) A mixture of aqueous humor and 20% desiccated embryo extract results in extensive proliferation of chick fibroblasts which is superior to that obtained in media prepared from desiccated horse serum, embryo extract and saline. (3) The possible explanations and significance of these findings have been discussed.

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Antithyrotoxic Effects of Antibiotics in Rats.* (18918)

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Several antibiotics have recently been shown to exert pronounced growth effects when fed in small quantities to chicks(1,2), pigs(3,4), or rats(5). These effects can be obtained on diets either adequate or deficient in vit. B_{12}(2,6), indicating that several factors may be made available through the action of the antibiotics. Ershoff(7,8) recently reported that supplements of dried penicillin mycelia or aureomycin mash, but not crystalline aureomycin HCl, prolonged the average survival rate of immature rats fed massive doses of thyroid powder. Since vit. B_{12} has been shown to be effective in counteracting the growth-retardation induced in young rats by administering large amounts of thyroid-active substances(9-11), it was of interest to see whether crystalline penicillin, neomycin or streptomycin would be similarly effective on rations either adequate or deficient in vit. B_{12}.

Procedure. In the first experiment, 50 young female albino rats (Carworth) were fed the following basal ration for 36 days: yellow corn meal, 35%; ground wheat, 25%; whole milk powder (Borden), 20%; linseed oil meal, 10%; alfalfa leaf meal, 6%; brewers yeast, 3%; and table salt, 1%. This ration is believed to contain an adequate supply of vit. B_{12},† and only one shipment of whole milk powder during the past 2 years was found to

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TABLE I. Effects of Protamone and Antibiotics on Body Growth, Food Intake and Oxygen Consumption of Young Female Rats on Vitamin B_{12}-Adequate Diet.

Treatment	No. of rats Orig	Final	Avg body wt, g Orig	Final	Gain	Avg food intake, g Total	Daily*	Per g gain in body wt	Avg O_2 consump/hr/ 100 g body wt, ml
1. Controls	10	10	145† (135-151)	181† (168-190)	36.5	393.8	10.9	10.8	179.5 ± 6.5‡
2. Protamone	10	9	133 (128-147)	155 (114-174)	22	484.1	13.5	22	312.8 ± 32.9
3. Protamone + streptomycin	10	9	139 (130-148)	164 (118-170)	24.3	492.5	13.7	20.2	280.4 ± 30.7
4. Protamone + penicillin	10	10	144 (129-150)	185 (156-195)	41	548.6	15.2	13.3	284.7 ± 19.8
5. Protamone + neomycin	10	9	137 (128-148)	188 (160-194)	50.5	550.8	15.3	10.9	272.5 ± 21.2

* Corrected for deaths.
† Weights expressed to nearest whole number.
‡ Standard error of mean.

be low in vit. B_{12}(12,13). This ration was supplemented with 0.16% Protamone‡ and either procaine penicillin G, streptomycin sulfate or neomycin sulfate§ in amounts of 0.05%. Body weight and food intake data were collected on alternate days. At the end of the experiment, basal oxygen consumptions were determined for each rat in a closed circuit Regnault-Reiset apparatus of the type described by Brody (14) and modified by Dr. E. P. Reineke of this department. The rats were fasted 15 hours prior to each test, and oxygen consumption was measured for 1½ hours. This procedure was repeated after 48 hours and the two values obtained for each rat were averaged. In all cases, there was less than 5% difference between the two figures obtained for each rat.‖ In the second experiment, 50 young male albino rats (Dixboro) were fed for 42 days the same ration as used in the previous experiment, except that soybean oil meal¶ was substituted for the whole milk powder. This ration, therefore, was free of animal protein and presumably of vit. B_{12}.** On the 42nd day, the rats were killed and the adrenals, spleen and thymus were removed and weighed. All rats were housed in wire bottom cages, in an air conditioned room at a temperature of 75 ± 1°F.

Results. The data from the first experiment are given in Table I and Fig. 1. The control rats (Group 1), which received neither Protamone nor antibiotic, gained an average of 36.5 g each and consumed an average of 10.9 g of food daily. The rats which received Protamone alone (Group 2), gained only an average of 22 g despite the greater average daily food intake. The efficiency of body-weight gains was reduced to about half, since about twice as many g of food were consumed

† Numerous generations of rats have been reared on this ration during the past 15 years at Michigan State College.

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‡ Protamone is the commercial name of an iodinated casein product containing thyroxine, and was kindly supplied by Cerophyl Labs., Kansas City, Mo.

§ The authors wish to express their appreciation to Dr. D. F. Green of Merck and Co., Rahway, N. J., for the procaine penicillin G, and to Dr. Gordon Stocking of The Upjohn Co., Kalamazoo, Mich., for the neomycin sulfate and streptomycin sulfate.

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‖ The authors wish to express their appreciation to Miss Y. S. L. Feng for her careful work in determining the oxygen consumption of these rats.

¶ Low fiber, solvent extracted, containing 50% protein. Manufactured by Archer-Daniels-Midland Co., Minneapolis, Minn.

** The addition of vit. B_{12} to this ration considerably enhances the growth rate of both strains of rats used in this study.

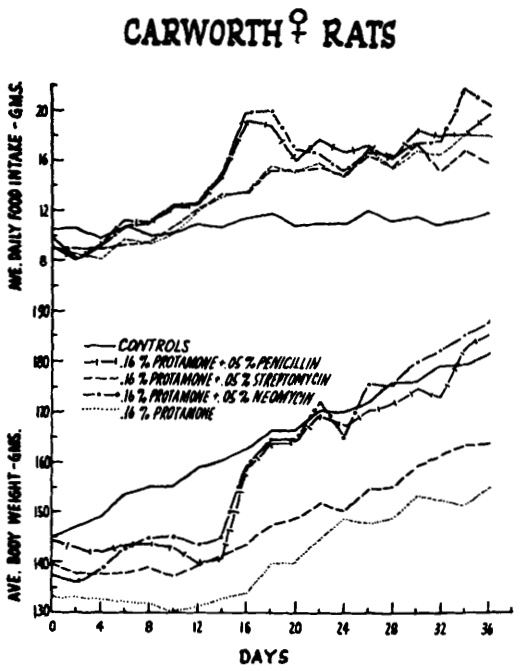

FIG. 1. Effects of Protamone and antibiotics on body growth and food consumption of female rats.

for each g of increment in body weight. The average basal oxygen consumption of these rats was increased 74% above the controls, indicating the presence of a severe degree of hyperthyroidism.

The addition of streptomycin sulfate (Group 3) apparently did not alter the results obtained with Protamone alone. However, procaine penicillin G (Group 4) or neomycin sulfate (Group 5) completely counteracted the Protamone-induced retardation of growth. The increase in food intake and body weight occurred rather abruptly, beginning on or about the 14th day of the experiment. The efficiency of body-weight gains was increased considerably above the Protamone-treated rats. Neither antibiotic significantly counteracted the increase in basal oxygen consumption induced by the Protamone.

The results of the second experiment (Table II), in which the animals were fed a diet presumably deficient in vit. B_{12}, were essentially similar to those obtained in the previous experiment. However, there were 3 notable exceptions. First, the antibiotics were relatively less effective in overcoming the inhibition of growth induced by the Protamone. Not only did the antibiotics fail to completely counteract the growth retardation, but it took 26 to 28 days before they showed any favorable effects on growth at all. The fact that the ration was deficient in vit. B_{12} may account to some degree for this reduced effectiveness of the antibiotics. Secondly, streptomycin sulfate was at least partially effective in increasing body weight, in contrast to its complete ineffectiveness in the first experiment. Finally, the mortality rate was increased by the antibiotics. All deaths occurred before the antibiotics exerted any favorable effects on growth.

The following data were obtained on the organ weights of the male rats. The adrenals of the controls averaged 14.1 $\pm$ 2.2 mg per 100 g of body weight, while those of the Protamone-treated rats averaged 38 $\pm$ 7.5 mg. This increase in adrenal weight is typical of hyperthyroid rats. Spleen weight was not altered either by Protamone alone or when combined with any of the antibiotics. Thymus weight in the controls averaged 160.1 $\pm$ 16.2 mg per 100 g of body weight and 107.1 $\pm$ 17.7 mg in the Protamone-treated rats, indicating an increased release of steroids from the adrenal cortex. Streptomycin and penicillin partially and neomycin completely counteracted the Protamone-induced reduction in thymus weight.

Discussion. These data indicate that crystalline penicillin, neomycin or streptomycin can partially or completely counteract growth retardation in young rats given large doses of Protamone. At least 2 of the antibiotics, penicillin and neomycin, appeared to be more effective in the presence of adequate amounts of vit. B_{12}, as suggested in the first experiment. Although all 3 antibiotics were at least partially effective on the ration deficient in vit. B_{12}, as seen in the second experiment, the possibility cannot be ruled out that these rats obtained some animal protein factor from the fecal material seen adhering to their tails or on the food containers. There may also have been a sex difference in the response of the rats to the antibiotics.

It is interesting that normal growth can

TABLE II. Effects of Protamone and Antibiotics on Body Growth and Food Intake of Young Male Rats on Vitamin B_{12}-Deficient Diet.

Treatment	No. of rats		Avg body wt, g			Avg food intake, g		
	Orig	Final	Orig	Final	Gain	Total	Daily*	Per g gain in body wt, g
1. Controls	10	9	130† (124-135)	246† (217-281)	115.9	612	15	5.3
2. Protamone	10	8	130 (126-136)	161 (126-196)	31.8	823.6	22.3	26.1
3. Protamone + streptomycin	10	6	129 (125-134)	182 (136-233)	53.4	861.4	26.9	16.1
4. Protamone + penicillin	10	6	129 (124-134)	196 (163-207)	66.8	887.8	25.9	13.3
5. Protamone + neomycin	10	7	129 (124-135)	178 (157-232)	49.2	839.6	23.1	17

* Corrected for deaths.
† Weights expressed to nearest whole number.

proceed in severely hyperthyroid rats in the presence of adequate amounts of vit. B_{12} and/or antibiotics. The great increase in basal metabolism effected by the Protamone, as indicated by the 74% increase in oxygen consumption, was not reduced significantly by the antibiotics. However, the antibiotics, like vit. B_{12}(15), increased both daily food consumption and ability to convert food into body-weight gains. The "thyrotoxicosis" of severe hyperthyroidism may largely be the manifestations of a multiple vitamin deficiency, as previously suggested(7).

The failure of the antibiotics to alter the increase in adrenal weight induced by the Protamone can be considered as evidence that they do not alter the turnover of Protamone in the body. Similarly, vit. B_{12} is not believed to increase the metabolism of Protamone or endogenous thyroxine in the rat(13,15). The antibiotics appeared to exert a partial or complete protective action on the thymus gland against atrophy by the Protamone. It is interesting that Pentz *et al.*(16) also reported that liver and vit. B_{12} partially counteracted the loss in thymus weight of thyroid-fed rats having greatly hypertrophied adrenal glands. They suggested that the protective effects of the liver and vit. B_{12} were exerted directly on the thymus gland.

Summary. (1) Three crystalline antibiotics were fed to 100 male and female rats to determine whether they could counteract the retardation of growth induced by feeding thyrotoxic amounts of Protamone. On a ration adequate in vit. B_{12}, penicillin or neomycin but not streptomycin were completely effective in overcoming the growth-inhibition, while on a ration deficient in vit. B_{12}, all 3 antibiotics were only partially effective. On the deficient ration, the antibiotics reduced the survival rate of the hyperthyroid rats. (2) At least part, if not all, of the effectiveness of the antibiotics in counteracting growth retardation could be attributed to the increase in food consumption and to an increased efficiency in converting food into body weight gains. The antibiotics did not alter the 74% increase in basal oxygen consumption induced by the Protamone, nor did they change the action of the latter on adrenal and spleen weight. Thymus atrophy was partially or completely prevented by the antibiotics.

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