day, the exudate which had accumulated in the carrageenin-treated animals was emptied and 4 ml of the above-mentioned trypsin solution was introduced into the air-sacs in both groups. With this doubled dose of trypsin, 80% of the controls showed necrosis of the skin above the pouch, while all the carrageenin-pretreated animals were perfectly protected. It may be concluded that an inflammatory granuloma, produced by an aqueous solution of an irritating polysaccharide, also gives protection against this enzyme.

Summary. Using the granuloma-pouch as an indicator, it has been shown that trypsin, given simultaneously with croton oil, causes particularly intense and widespread tissue-damage in the rat. On the other hand, if trypsin is introduced into the inflamed area 5 days after the croton oil, the granulomatous lining, formed under the influence of this chemical irritant, protects the adjacent tissues against otherwise toxic concentrations of this enzyme. The importance of the time-factor, in determining the effect of trypsin upon inflamed tissue, is emphasized.

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Received February 11, 1954. P.S.E.B.M., 1954, v86.

A Possible Role of Iodinated Casein in Intestinal Assimilation of Vitamin B_{12}.* (20996)

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A widely used technic for increasing responses of animals to dietary vitamin B_{12} is the dietary inclusion of iodinated casein, as outlined by Betheil *et al.*(1), and others(2,3). It apparently is generally considered that the iodinated casein is effective because of its content of thyroxine and related compounds (4,5), which by increasing the rate of metabolism of a vit. B_{12}-deficient diet, makes animals thus conditioned more sensitive to dietary vit. B_{12}. That this may not be the whole explanation is indicated by the present results.

Experimental. Fifty day-old single comb White Leghorn cockerels were placed, after one day on corn meal, on a corn-soy diet† containing no animal products. An equal number was placed on this diet to which had been added 0.05% of iodinated casein.‡ Both diets contained 1.0% of formylsulfathiazole.§ Half of the birds under each dietary regimen were subjected to a 32-hour fast at 2 days, and again at 8 days of age, in an attempt to deplete yolk-sac reserves. Thus there were 4 experimental treatments in the depletion phase of the experiment: 1) basal, 2) basal plus fasting regimen, 3) basal plus iodinated casein, and 4) basal plus iodinated casein plus fasting regimen. At age 12 days, 5 birds, and again at age 15 days 4 birds were taken from each group for measurement of the experimental criteria. Each group then contained 16 residual birds. These were divided into halves. One half from each depletion treatment was placed on the same B_{12}-deficient basal diet‖

* Scientific Series Paper No. 433, Colo. Agr. Exp. Station. Supported in part by Merck and Co., Rahway, N. J.

† The same as described previously(6).

‡ Protamone, kindly supplied by Dr. W. R. Graham, Cerophyl Laboratories, Kansas City, Mo.

§ Formo-Cibazol, manufactured by Ciba Pharmaceutical Products, Inc., provided through the courtesy of Dr. F. X. Gassner.

TABLE I. Average Body Weights (g).

Treatment	Depletion period 6/27 (Initial)	Depletion period 7/11		B_{12} comparison period 7/11	B_{12} comparison period 7/27
Basal	41 (25)	108 (20)	a	113.5(8)	285 (8)
			b	107.5(8)	282 (8)
Fasted	41 (25)	102 (21)	a	107 (8)	265.5(8)
			b	99.4(8)	254 (7)
Protamone	41 (25)	116 (20)	a	105 (8)	257.5(8)
			b	127 (8)	295.5(8)
Fasted with Protamone	41 (25)	96.2(19)	a	92.4(8)	253 (8)
			b	104 (7)	287 (7)

The "a" subgroups were continued after 7/11 on basal diet. The "b" subgroups were given this diet plus 25 μg B_{12}/kg. All differences in weight between corresponding "a" and "b" subgroups are due to selection that date, and should be kept in mind in studying weights of 7/27. Numbers in parentheses tell the number of bird weights averaged.

as used for depletion, the other half on this diet to which had been added 25 μg of crystalline vit. B_{12}¶ per kg of diet. This afforded a chance to observe response to vit. B_{12} by chicks from all 4 depletion regimens. After 2 weeks on the B_{12} response trial (at age 4 weeks) all birds were sacrificed for termination samples. Criteria determined on the termination samples were 1) body weight; 2) liver weight; 3) vit. B_{12} content of a) liver, b) small intestine plus contents,** and c) ceca plus contents; 4) and 5) coliform counts and yeast-mold counts on a) small intestine plus contents,** and b) ceca plus contents. In addition body weight, liver weight and liver vit. B_{12} were determined for the depletion samples, as well as microbial counts on excreta at 18 days of age.

Results. The results are shown in Tables I-III. It is apparent from Table I that significant growth responses to vit. B_{12} were not obtained. Liver weights were observed, but were not markedly affected by any of the treatments, except as would be expected as a result of fasting. Yet in Table II large differences in liver vit. B_{12} appear. The inference must be that the levels of liver vit. B_{12} found in the non-B_{12} chicks corresponded to adequate or more than adequate nutrition with respect to vit. B_{12}. This is in keeping with earlier experience(6). Furthermore the great increase in liver B_{12} potency against time, even in the non-B_{12} chicks, indicates that biosynthesis of vit. B_{12} was taking place in all groups.

The supposed biosynthesis might well be questioned, since in this experiment all diets contained 1% of formylsulfathiazole, which was recommended as an intestinal microbial deterrent. It is clear from Table III, however, that the ceca from birds in all treatments were well supplied with coliform, yeast, and mold organisms. (Similar counts on small intestine gave uniformly negative results.) Furthermore (Table II) the vit. B_{12} contents of the ceca were much higher than those of the small intestines. From these results it can scarcely be doubted that intestinal biosynthesis of vit. B_{12} was taking place; although the development of liver concentration of vit. B_{12} against time (Table II) supports the supposition that the sulfa compound may at first have been quite effective in reducing biosynthesis, but later lost its effectiveness, probably by the development of resistant strains among the microflora.

It would appear (Termination, Table II) that Protamone increased responsiveness of chicks to vit. B_{12} in terms of liver content of the vitamin. This effect may have been partly

‖ Birds depleted in the presence of Protamone continued to receive it. Birds depleted in the absence of Protamone did not now receive it.

¶ Through the courtesy of Dr. L. Michaud, Merck and Co., Rahway, N. J.

** Approximately 5 inches beginning at, and running posteriorly from the pancreatic duct. These, and the ceca plus contents were prepared for plate counts and vit. B_{12} assay by preliminary maceration, in sterile Waring blendors, of dilutions made in physiological saline solution.

TABLE II. Average Vitamin B_{12} Contents of Tissues.

	Depletion				Termination				
	7/8		7/11		7/27				
Treatment*	mμg B_{12} /g liver	mμg B_{12} /liver	mμg B_{12} /g liver	mμg B_{12} /liver		mμg B_{12} /g liver	mμg B_{12} /liver	mμg B_{12} /g cecum†	mμg B_{12} /g small intestine†
Basal	6.04	21.7	9.19	38.9	a	194	1410	212	12.6
					b	437	3320	190	28.5
Fasted	4.36	12.1	14.8	51.5	a	190	1260	124	15.9
					b	433	2700	129	25.6
Protamone	8.00	27.6	11.4	59.4	a	111	756	150.5	17.1
					b	465	3310	342	24.0
Fasted with Protamone	4.06	8.28	23.75	81.9	a	122	756	121	17.9
					b	374	2603	412	24.05

* Treatment shown was prior to subdivision for B_{12} response trial. "a" subgroups were controls, "b" subgroups B_{12} supplemented.
† Including contents.

a negative one, since in the non-B_{12} birds liver vit. B_{12} was reduced by Protamone from levels found in the controls. The effects within the gastrointestinal tract are seen in Table II. Note that insofar as the small intestine is concerned, the vit. B_{12} contents reflected dietary supply. Where the ceca are concerned, however, this relationship was lost *except in chicks given Protamone.* In these the ceca plus contents contained much more vit. B_{12}, consequent to dietary inclusion, than did the controls (also given vit. B_{12}, but without Protamone). This was true whether or not the birds had previously been fasted. Since it was *not* true in the absence of Protamone, the best hypothesis to account for the results is that Protamone interferes with intestinal absorption of vit. B_{12}.††

Discussion. The fact that reduction in liver B_{12} due to Protamone occurred only in the absence of added dietary B_{12} is of interest, and of possible significance in view of other work. Allen *et al.*(7), working with young dairy animals, found reduced plasma levels of carotenoids and vit. A as a consequence of feeding iodinated casein; *but only at a low carotene intake.* The effect was not detectable in other animals on a liberal carotene intake, Robblee *et al.*(3) pointed out that the effects obtained from thyroactive materials may be greatly conditioned by marginal dietary deficiencies. The thyrotoxicosis observed by them and by others in chicks at sub-marginal levels of the factor in liver anti-pernicious anemia extract and fish solubles (vit. B_{12}) may have been due to an aggravation of the deficiency of that factor by the thyroactive agents. The present results lend further support to this explanation, and suggest mechanisms by which the aggravation may be accomplished.

While the results of this experiment are not extensive, and therefore not conclusive, they shed new light on the reasons for the effectiveness of iodinated casein in rendering animals responsive to vit. B_{12} in their diets. This effectiveness has long been recognized, but never entirely understood. From the data here recorded, it appears that we must look elsewhere than to the system of endocrine interactions to fully account for the existing state of affairs. Further work along these lines is contemplated.

Summary. Dietary iodinated casein led in

†† Another possible explanation of the results must not be overlooked. This would involve two suppositions, namely (a) that Protamone under some conditions stimulates intestinal biosynthesis of vit. B_{12} in portions of the gastro-intestinal tract from which the resulting vitamin is not assimilated, and simultaneously (b) the familiar assumption that Protamone by metabolic stimulation depletes tissue (liver) vit. B_{12}; this effect being observable only at marginal nutritional status. In the absence of precise knowledge regarding assimilation of nutrients from the lower tract in chicks, liver storage capacity for vit. B_{12}, and ease of digestion and assimilation of Protamone, it is difficult to give a clear preference to either explanation suggested.

TABLE III. Microbial Counts.

Treatment*		(7/14) Excreta Coliform, cells/g × 10^6	(7/14) Excreta Yeast-mold, cells/g × 10^4	Termination (7/27) Ceca with contents Coliform, cells/g × 10^7	Termination (7/27) Ceca with contents Yeast-mold, cells/g × 10^5
Basal	a	11.0	125	2.23	2.86
	b	4.3	.24	1.18	4.09
Fasted	a	1.3	3.7	2.21	2.95
	b	.75	1.4	.385	1.52
Protamone	a	3.6	1.0	3.50	2.12
	b	15.0	1.1	.931	4.22
Fasted with Protamone	a	1.3	.18	1.24	2.09
	b	1.7	20.0	1.40	—

* Treatment shown was prior to subdivision for B_{12} response trial. "a" subgroups were controls, "b" subgroups B_{12}-supplemented.

chicks given dietary vit. B_{12} to higher cecal contents of vit. B_{12}; and in chicks not given dietary vit. B_{12} to lower liver contents of vit. B_{12} than those found in respective controls. It is suggested that the ability of iodinated casein to increase the sensitivity of chicks to dietary vit. B_{12} is due in part to an interference with intestinal absorption of the vitamin.

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Received March 2, 1954. P.S.E.B.M., 1954, v86.

Comparison of Methods for Recovering Poliomyelitis Viruses from Human Sources.*† (20997)

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The substitution of new methods for old requires an appraisal of their comparative merits. A large number of published papers attest to the usefulness of tissue culture methods for growth and immunological identification of poliomyelitis viruses, and for serum antibody surveys in the human population (1,2). In many studies stock viruses were used after adaptation to tissue culture from monkey or rodent sources. A few studies (3-6) have explored the usefulness of tissue culture methods in recovering poliomyelitis virus in specimens obtained directly from human beings.

In casting out among several methods most amenable for use in our laboratory it was quickly evident that some comparative information was required if confidence were to be

* Aided by a grant from the National Foundation for Infantile Paralysis.

† Acknowledgement is made to Dr. James Mott, Division of Epidemiology, Kansas State Board of Health, Topeka, Kan., for assisting in collection of some of specimens.