

Conclusion. From the observations made on the chemical and immunological studies of the polysaccharide, lipoid, and protein fractions obtained from representative serological and cultural types it is justifiable to conclude that the alkali-soluble protein is the cellular constituent responsible for type-specificity in *C. diphtheriae*.

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Thyroid Enlargement Following Liver Feeding in Rats.

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That the feeding of liver to animals produces an enlargement of the thyroid gland was reported by Hunt,¹ who found that the thyroid of mice fed on a diet of oatmeal and liver was larger and more vascular than of those on a diet of eggs, crackers and milk. Marine² produced a hyperplasia of the thyroid in brook trout by feeding them with liver and heart. Burget³ found that rats kept under hygienic conditions and fed a high protein diet, consisting of fresh liver and lean beef mixed with a little oatmeal or bread crumbs, developed a hyperplasia of the thyroid. All the above reports of enlargement of the thyroid were interpreted as due to the high protein diet.

Recently, Remington⁴ reported that in a low iodine goiter-producing diet, the replacement of a part of the wheat gluten by dried pig liver aggravated the degree of goiter, while purified casein prevented goiter formation, and yeast had no effect. This led Remington to believe that the liver in his goiter-producing diet was responsible for the enlargement of the thyroid. The writer (Hou⁵) in the same year reported that rats fed on a soybean-millet diet with dried liver powder as the only source of animal protein developed a marked hypertrophy of the thyroid gland. The enlargement was over 10 times when the animal was fed on the millet and liver diet for over a year. It becomes of great interest to know which constituent or constituents

¹ Hunt, Reid, *J. Am. Med. Assn.*, 1911, **57**, 1032.

² Marine, D., *J. Exp. Med.*, 1914, **19**, 70.

³ Burget, G. E., *Am. J. Physiol.*, 1917, **44**, 492.

⁴ Remington, R. E., *J. Nutrition*, 1937, **13**, 223.

⁵ Hou, H. C., *Chinese J. Physiol.*, 1936, **10**, 659; 1937, **12**, 488.

of the diet is or are responsible for the enlargement of the thyroid. The following series of experiments were accordingly carried out.

Albino rats of the Wistar strain were selected with reference to litter mates and sex when about one month old. Five rats, each from a different litter, were put into one group. The litter mates were put into 3 other groups. The 4 groups were given 4 different diets as follows:

A7	Soybean Flour	450	A7a	Soybean Flour	450
	Millet	500		Millet	500
	Sodium Chloride	20		Sodium Chloride	20
	Dried Ox Liver Powder	30		Dried Beef	30
				Carotene (0.008 mg per rat per day)	
				Ostelin	2 cc
A7b	Soybean Flour	450	A7c	Soybean Flour	450
	Millet	500		Millet	500
	Sodium Chloride	20		Dried Beef	30
	Dried Ox Liver (extracted with Alcohol)	30		Sodium Chloride	20
	Carotene (0.008 mg per rat per day)			Residue of Alcohol Extract of Ox Liver (from 30 g of dried liver)	
	Ostelin	2 cc		Ostelin	2 cc

The rats were kept on these diets for 3 months and then sacrificed. The thyroid glands were weighed with a micro-analytical balance immediately upon their removal from the body. The weights of thyroid glands of the various groups are shown in Table I.

It will be noted that rats fed on diet A7 had the largest thyroid, next rats on A7c, then those on A7a. Rats fed on A7b had the smallest thyroid which is within the normal range of thyroid weight. From these results it appears that the dried ox liver was the constituent in the diet which caused the marked enlargement of the thyroid since the substitution of liver by dried beef resulted in only a slight

TABLE I.
Influence of Liver and Liver Extract on Thyroid Gland.

	A7 (Dried ox liver)		A7a (Dried beef)		A7b (Extracted liver)		A7c (Alcoholic extract of liver)	
	Body wt g	Thyroid mg	Body wt g	Thyroid mg	Body wt g	Thyroid mg	Body wt g	Thyroid mg
	127	58.7	96	14.8	57	9.2	110	31.8
	98	53.8	88	19.0	150	16.0	120	38.3
	138	55.5	93	24.3	164	17.0	130	35.4
	108	33.8	156	42.9	158	13.5	140	50.0
	90	29.7	113	23.5	147	18.1	111	28.3
Avg	112	46.3	109	24.9	135	14.8	122	36.7
Thyroid mg/100 g Body wt.	41.3		22.8		10.9		30.1	

enlargement of the thyroid. Furthermore, when the liver had been extracted with alcohol (diet A7b), its feeding did not induce any enlargement of the thyroid. On the other hand, the inclusion of the residue from the alcoholic extract of liver in the diet containing dried beef (A7c) caused the thyroid to increase in size to a degree approximating the enlargement due to the dried liver diet (A7).

It appears, therefore, that the factor which causes an enlargement of the thyroid in rats lies in the alcohol-soluble portion of the dried liver. The enlargement as shown by histological examination was general in character with the organ a little more vascular and cellular than normal. Further experiments are being carried out to determine the actual substance present in the alcohol-soluble portion of liver which causes the enlargement of the thyroid.

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Cultivation of Virus of Encephalitis (St. Louis Type) on Agar Tissue Medium.

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Although the virus of encephalitis of St. Louis type has been successfully cultivated by Maitland technic and on the chorio-allantoic membrane of developing chick¹⁻⁴ the titer obtained in these cultures has been invariably low. None of the cultures showed a titer as high as 10^{-6} even after repeated passages. Recently Kawakita⁵ has reported successful cultivation of Japanese encephalitis virus in a special medium containing chick allantoic fluid. It has occurred to us to attempt to cultivate the virus of St. Louis type on the agar-tissue medium which has been found to be satisfactory for the growth of various types of rickettsiae.^{6, 7} The results of such a study together

¹ Syverton, J. T., and Berry, G. P., *Science*, 1935, **82**, 596.

² Harrison, R. W., and Moore, E., *Am. J. Path.*, 1937, **13**, 361.

³ Schultz, E. W., Williams, G. F., and Hetherington, A., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 799.

⁴ Smith, M. G., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 191.

⁵ Kawakita, Y., *Jap. J. Exp. Med.*, 1939, **17**, 211.

⁶ Zinsser, H., FitzPatrick, F. K., and Wei, H., *J. Exp. Med.*, 1939, **69**, 179.

⁷ Zia, S. H., Liu, P. Y., and Pang, K. H., *Chinese Med. J.*, 1938, **54**, 547.