had disappeared; and rain seeped through the leaky roofs of the desolate buildings. But President Ewell still rang the bell. It was an act of faith. It was a gesture of defiance. It was a symbol of determination that the intellectual and cultural tradition must be kept alive, even in a bankrupt world.

In every school, college and university of America to-day we need to hear that bell ringing.

SPECIAL ARTICLES

THE RELATIONSHIP OF VITAMIN A TO RESISTANCE TO NIPPOSTRONGYLUS MURIS

A CRITICAL study of the work of Spindler[1] revealed the fact that lack of vitamin A in the diet lowered the resistance of albino rats to a superinfection with the nematode, *Nippostrongylus muris;* but details were lacking as to the composition of the diet or the extent of the vitamin A deficiency in the experimental animals. It seemed necessary that further work should be done, by methods somewhat similar to those employed by McCoy[2] with *Trichinella spiralis,* and by Lawler[3] with *Strongyloides ratti;* these workers correlated vitamin A deficiency with susceptibility.

It is the purpose of the present paper to give the findings obtained under controlled diet, and with chemical determination of liver vitamin A, on the influence of vitamin A depletion on the resistance of the pied stock rats, McCollum strain, to primary infection and subsequent reinfections with *Nippostrongylus muris.*

In carrying out the tests referred to in this paper, the experimental and control animals were divided into groups such that sex and weight distribution were fairly uniform. The rats averaged 70 to 80 grams in initial weight. The experimental groups were fed, ad lib, two different diets deficient in vitamin A: Diet I the yeast diet and Diet II the "synthetic" diet, respectively; and the control groups were fed the same diets plus a supplement of vitamin A, such that each rat received 150 I. U. per week. The supplement was a vitamin A concentrate diluted with sesame oil, and fed in three weekly doses by medicine dropper. The composition of the two diets will be reported in detail later.

Chemical Methods: The vitamin A content of the liver was used as an index of relative depletion. The entire liver was excised, weighed and ground up with anhydrous Na_2SO_4 until a uniform powder was obtained. An aliquot portion of liver powder was extracted with 15 cc of petroleum ether in a test tube; after centrifuging, a 10 cc portion of this extract was transferred to an Evelyn photoelectric colorimeter absorption tube. The extract was evaporated to dryness in a stream of dry CO_2, with the tube immersed in a water bath at 55–60° C. The residue in the tube was taken up in 1 cc of chloroform, and the vitamin A content determined by the addition of 9 cc of Carr-Price reagent (25 gms of $SbCL_3$ in 100 cc of $CHCL_3$), with the colorimetric measurement of the blue color developed in the Evelyn photoelectric colorimeter. The blood vitamin A was determined by the method of Kimble.[4]

Parasitological Methods: Infective larvae (isolated by means of Baermann's apparatus from 9–10 day charcoal cultures of feces of infected rats) were counted by the dilution method, suspended in known volumes of water, and injected subcutaneously into the rats to be infected. At subsequent autopsy, worms found in the intestines were counted. To determine the number of larvae in the lungs, these organs were removed at autopsy and cut into small pieces, pressed between two plate-glass slides (2″ × 3″) and examined microscopically for the presence of larvae.

RESULTS

Experiment 1. The effect of vitamin A depletion upon susceptibility to primary infection. After being on the stated diets for 56 days, the average weight of the 24 rats reached about 140 grams. On the 57th day, each of these animals was given 2,300 *N. muris* larvae, and was sacrificed 12 days later. Post-mortem determinations revealed a complete depletion of vitamin A in the livers, and low vitamin levels in the blood, of the animals receiving vitamin A-deficient diets. These animals harbored more worms in the intestines than those on the same diets supplemented with vitamin A concentrate. It will be noted further that three rats fed on Diet I-a and two rats fed on Diet II-a died on the fifth and sixth days following infection with the larvae; whereas all the control animals, though given the same number of larvae, survived (see Table 1).

Experiment 2. The effect of vitamin A depletion upon susceptibility to subsequent reinfections. Rats, the average weight of which was between 70 and 75 grams, were "hyperimmunized" by means of serial infections with 2,000 larvae each. The first infection ran its course for two weeks; 2,000 additional larvae were then administered, followed by a third infection of 2,000 larvae two weeks later. Three weeks after the last infection the animals were divided into groups, according to the diets administered.

After being on the experimental diets for 79 days, the average weight of the 22 rats reached about 180

[1] L. A. Spindler, *Jour. Parasit.,* 20: 72, 1933.
[2] O. R. McCoy, *Amer. Jour. Hyg.,* 20: 169, 1934.
[3] H. J. Lawler, *Am. Jour. Hyg.,* 34 (Sec. D): 65, 1941.
[4] M. S. Kimble, *Jour. Lab. Clin. Med.,* 24: 1055, 1939.

grams. On the 80th day, each animal was given 2,000 *N. muris* larvae and was sacrificed 12 days later. Post-mortem determinations revealed a complete depletion of vitamin A in the liver and low vitamin A levels in the blood of the animals on the vitamin A-deficient diets. More worms were found in the intestines, but fewer worms in the lungs, of these animals; in the control animals the reverse condition was seen. See Table 1.

TABLE 1

Experiment	Dietary group	Blood Vit. A μ gm. per cent.	Liver Vit. A μ gm. per liver	Worms found at autopsy: No. in intestine	Worms found at autopsy: Mean per cent. infection	Worms found at autopsy: In one lung
I.	*Diet I*					
	a. (− Vit. A)*	4.8	5.5	2013.33‡	87.53	
	b. (+ Vit. A)*	29.3	215.0	1656.50	72.01	
	Diet II					
	a. (− Vit. A)*	6.3	3.4	2201.25§	95.77	
	b. (+ Vit. A)*	21.8	271.5	1654.17	71.92	
II.	*Diet I*					
	a. (− Vit. A)†	6.3	7.8	159.40	7.97	27.00
	b. (+ Vit. A)†	27.5	205.56	12.60	0.63	72.20
	Diet II					
	a. (− Vit. A)*	5.6	8.4	204.33	10.22	23.8
	b. (+ Vit. A)*	22.7	310.98	13.83	0.69	59.5

* Represents mean values for 6 animals.
† Represents mean values for 5 animals.
‡ Represents mean values of 3 surviving animals.
§ Represents mean values of 4 surviving animals.

Experiment 3. The effect of vitamin A depletion upon the passive transfer of immunity. In order to test the influence of vitamin A deficiency upon the efficacy of plasma from "hyperimmunized" rats, each of the two groups of normal rats, the average weight of which was about 75 grams, was infected with approximately 600 larvae, and concurrently was administered an intraperitoneal dose (5 cc per 100 gms body weight) of plasma. When the animals were sacrificed 12 days later, post-mortem determinations revealed that animals that had been given plasma from "hyperimmunized" rats fed on vitamin A-deficient diets had more worms in the intestines (average 319.33 worms per rat) and fewer worms in the lungs; while reverse conditions existed in rats given plasma from "hyperimmunized" rats fed on adequate diet with supplementary vitamin A (average 168.6 worms per rat in intestines, with more worms in the lungs).

Conclusions

The above results indicate that the lack of vitamin A in the diet of the experimental animals lowers their resistance to primary infection, as well as subsequent reinfection, with *Nippostrongylus muris*. Furthermore, plasma derived from animals with low vitamin A levels affords no protection against this parasite in the way of positive transfer of immunity. In contrast, the rats fed on the same diet, plus vitamin A supplement, developed a marked resistance to infection with this nematode, such as has been described by Schwartz *et al.*,[5] in addition to protection rendered normal rats by plasma from "hyperimmunized" rats, as previously demonstrated by one of us.[6]

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STUDIES REGARDING A GLUTAMINE-LIKE SUBSTANCE IN BLOOD AND SPINAL FLUID

In view of the recent communication of Hamilton,[1] regarding the presence of a glutamine-like substance in the blood, it may be of interest to note some studies which we have been carrying on during the past few years regarding substances in the blood and spinal fluid which split off ammonia readily on mild acid hydrolysis.[2] We have found that spinal fluid, trichloracetic acid filtrates of blood plasma and serum, and ultrafiltrates of plasma and serum contain a substance which yields ammonia under the treatment indicated. It was also found that the rate of hydrolysis of this substance under a variety of conditions of acidity and temperature was practically identical with that of glutamine which we had prepared from beet root.[3] A quantitative method based upon those findings was developed which has been used up to date in a study of a rather large amount of clinical material and also in animal experiments.

The findings in our blood studies in man and rabbit are in keeping with those of Hamilton,[1] namely, that the amount of ammonia liberated is equivalent approximately to from 5 to 10 mg of glutamine per 100 cc of plasma or serum. It has also been found that spinal fluid contains similar amounts of this substance. This together with the studies with ultrafiltrates we believe is added evidence that the findings for blood filtrates are not due to artefacts resulting from its chemical treatment. Our method also rules out the possible presence of any appreciable amount of asparagine in the material studied.

It has been found further that insulin hypoglycemia and also the administration of glucose reduces the

[5] B. Schwartz, J. E. Alicata, J. T. Lucker, *Jour. Wash. Acad. Sci.*, 21: 259, 1931.
[6] J. Y. C. Watt, Abstract of Doctor's Thesis, Cornell University Press, 1942.
[1] P. Hamilton, *Jour. Biol. Chem.*, 145: 711, 1942.
[2] M. M. Harris, Tenth Annual Report of the Director of the New York State Psychiatric Inst. and Hosp., p. 41, 1940.
[3] H. B. Vickery, G. W. Pucher, H. E. Clark, *Jour. Biol. Chem.*, 109: 39, 1935; *Biochem. Jour.*, 29: 2710, 1935.