Hence the impaired metabolic mechanism of defectives evidently requires a larger relative amount of carbohydrates in their food. Within the organism carbohydrates circulate in the form of glucose. Therefore, our inference is that the circulating glucose is a factor in the metabolic mechanism and its effect increases with the increase of its quantity.

There is probably an increase in the quantity of circulating glucose in our patients, when after their last meal they rest comfortably in the living room. Apparently during this period glucose is not used as a source for muscular energy and is available as a factor in metabolism. Therefore, we ought to expect that in the urine collected from 5:00 P. M. there will be decreased quantity of the hydrolyzable products. Table V records the glycuresis in the urine collected from 5:00 P. M. to midnight. There is none or very little of post-hydrolysis reducing substances, which is in full conformity with the idea that glucose is a factor in metabolism.

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Protective Power of Serum of Animals Fed Pneumococci or Tissue of Animals Killed by Pneumococcus.

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In an earlier paper on the immunization of rats to pneumococcus, produced by feeding the tissues of animals killed by the same organism, brief mention was made of the fact that the blood of the immune animals contains protective antibodies.[1] The present article is a report of the experiments made with sera of rats and dogs. The rats were fed either the infected tissue, the living pneumococcus, or the acid-killed germ. Such animals have already been shown to possess immunity to intraperitoneal injection of virulent pneumococci.[2, 3] The dogs were fed either the living pneumococcus, or infected rabbit tissue. The object was to learn whether the serum

TABLE I.

Protective power of serum of rats immunized against pneumococcus, by feeding tissues of animals killed by the same organism. Mice were used in the tests. S = Survived. D = Died -days.

	Nov. 11/25			Nov. 19/25			Nov. 21/25			Nov. 23/25			Nov. 25/25			Dec. 4/25			Feb. 24/26		
Dose cc.	Pn. Alone	Pn. + Control Serum	Pn. + Immune Serum of Treated Rat No. 186	Pn. Alone	Pn. + Control Serum	Pn. + Immune Serum of Treated Rat No. 187	Pn. Alone	Pn. + Control Serum	Pn. + Immune Serum of Treated Rat No. 189	Pn. Alone	Pn. + Control Serum	Pn. + Immune Serum of Treated Rat No. 191	Pn. Alone	Pn. + Control Serum	Pn. + Immune Serum of Treated Rat No. 199	Pn. Alone	Pn. + Control Serum	Pn. + Immune Serum of Treated Rat No. 192	Pn. Alone	Pn. + Control Serum	Pn. + Immune Serum of Treated Rat No. 275
10^{-6}	S	D2	S	S	D2	S	D3	D2	D2	D3	D2	D10	D2	D2	S	D2	D2	D2	D3	D3	D3
10^{-7}	D2	D2	S	D2	D2	S	D2	D2	S	D2	D3	S	D2	S	S	D2	D2	D2	D2	D2	D2
10^{-6}	D2	D2	S	D2	D2	S			D2	D2	D2	D9		S	S	D2	D2	D2		D2	D4
10^{-5}	D2			D2	D2	D2			S	D2	D2	D3		D2	S	D1	D1	D2		D3	D2
10^{-4}				D2	D2	S			D2	D2	D2	D2		D2	D2	D1	D1	D1		D2	D2
10^{-3}				D2	D2	D3			S	D2	D2	D2									
10^{-2}				D2	D2	D2			D3	D2	D2	D2									
10^{-1}									D2	D2	D2	D2									

of such animals would protect mice against an intraperitoneal injection of virulent pneumococci. All experiments were done with type I. The preparation of the infected tissue, of the pneumococci, and their feeding, have been described elsewhere.[1, 2, 3]

To test an immunized rat's serum, the neck was shaved, the rat made unconscious by a blow across the head, the jugular vein cut, and the blood collected and centrifuged. A group of mice would be injected intraperitoneally, with graded doses of a 24-hour culture

TABLE II.

Protective Power of Serum of Dogs Fed Tissues of Rabbits Killed by Pneumococcus. S=Survived. D=Died -days.

	Feb. 2/26			Feb. 11/26			Feb. 15/26			Feb. 20/26		
Dose cc.	Pn. Alone	Pn. + Serum of Control Dog No. 6	Pn. + Serum of Treated Dog No. 1	Pn. Alone	Pn. + Serum of Control Dog No. 5	Pn. + Serum of Treated Dog No. 2	Pn. Alone	Pn. + Serum of Control Dog No. 7	Pn. + Serum of Treated Dog No. 3	Pn. Alone	Pn. + Serum of Control Dog No. 8	Pn. + Serum of Treated Dog No. 4
10^{-7}	D2			D3			S			D2		
10^{-6}		S	S	D1	D3	S	D2	S	S	D2	S	S
10^{-5}		D2	S		D3	S		D4	S		D4	S
10^{-4}		D2	S		D3	S		D3	D7		D6	S
10^{-3}		D2	D3		D3	D3		D3	D5		D2	D5
10^{-2}		D2	D2		D2	D2		D4	D2		D2	D3

TABLE III.

The protective power of the sera of Dogs 5, 6, 7, and 8 after they were fed live pneumococci. S=Survived. D=Died -days. The figure preceding D or S gives the weight of the mouse tested.

	Apr. 9/26				June 15/26			
Dose cc.	Pn. Alone	Pn. + Serum of Control Dog No. 10	Pn. + Serum of Treated Dog No. 5	Pn. + Serum of Treated Dog No. 6	Pn. Alone	Pn. + Serum of Control Dog No. 11	Pn. + Serum of Treated Dog No. 7	Pn. + Serum of Treated Dog No. 8
10^{-9}					22 S			
10^{-8}	S				22 S			
10^{-7}	D3 D3				24 D2	23 S	23 S	20 S
10^{-6}	D3 D3	S	S	S	25 D2	21 S	20 S	21 S
10^{-5}		S	S	S		21 D2	22 S	21 S
10^{-4}		D3	S	S		22 D3	22 S	21 D3
10^{-3}		D2	D4	D5		22 D2	23 D2	22 D2
10^{-2}		D2	D6	D3		21 D2	20 S	21 D3

TABLE IV.

Comparison of protective power of serum of rats immunized by feeding pneumococci (HCl killed or living) and by subcutaneous injection of dead bacteria. S = Survived. D = Died -days. The figure preceding D or S gives the weight of the mouse tested.

Dose cc.	August 30, 1926.					September 8, 1926.				
	Pn. Alone	Pn. + Control Serum	Pn. + Serum of Rat Immun. by feeding live Pn.	Pn. + Serum of Rat Immun. by feeding HCl killed Pn.	Pn. + Serum of Rat Immun. by Subcut. Inject.	Pn. Alone	Pn. + Control Serum	Pn. + Serum of Rat Immun. by feeding live Pn.	Pn. + Serum of Rat Immun. by feeding HCl killed Pn.	Pn. + Serum of Rat Immun. by Subcut. Inject.
10^{-9}	18 D2					18 S				
10^{-8}	22 D2					21 S	19 S	19 S	20 S	20 S
10^{-7}	24 D2	19 S	20 D2	21 S	21 S	21 S	21 S	21 S	21 S	21 S
10^{-6}	26 D2	22 D2	23 D2	23 S	23 S	23 D1	22 S	22 S	23 S	23 S
10^{-5}	28 D2	25 D2	26 D2	26 S	26 D2	26 D2	24 D2	24 S	25 S	25 D5
10^{-4}		26 D3	27 D2	27 D2	27 D2		26 D2	26 D2		27 D2
10^{-3}		28 D2	28 D2	28 D2	32 D2					
10^{-2}			32 D2	30 D2	38 D2					

Dose cc.	September 14, 1926.					September 21, 1926.				
10^{-9}	19 S					18 D3				
10^{-8}	21 S					20 D2				
10^{-7}	23 D2	19 D2	21 D2	21 D2	21 S	24 D2	18 D1	18 D2	18 D2	19 S
10^{-6}	24 D2	22 D2	22 D2	22 D2	23 S	25 D2	20 D2	21 D2	21 D2	24 S
10^{-5}	25 D2	23 D2	23 D2	23 D2	23 S	27 D2	25 D2	25 D2	25 D2	25 D3
10^{-4}		24 D2	25 D3	25 D2	25 D2		25 D2	26 D2	26 D2	26 D2
10^{-3}		23 D2	26 D2	26 D2	25 D2		27 D2	27 D2	27 D2	28 D2
10^{-2}			27 D2	30 D2	31 D2			29 D2	29 D2	32 D1

Dose cc.	October 1, 1926.					October 7, 1926				
10^{-9}	19 S					16 S				
10^{-8}	20 S					18 D2				
10^{-7}	20 D2	19 D2	19 D2	19 S	19 S	19 D2	16 D2	16 S	17 D2	18 D2
10^{-6}	22 D2	20 D3	20 D2	20 S	20 S	20 D2	19 D3	19 D2	19 D2	19 D3
10^{-5}	24 D2	21 D2	21 D2	21 D2	22 S	20 S	19 D2	19 D2	19 D3	19 D3
10^{-4}		22 D2	22 D2	23 D2	24 S		20 D2	20 D3	20 D2	20 D3
10^{-3}		26 D3	27 D2	27 D2	27 S		20 D2	20 D3	21 D2	21 D2
10^{-2}			27 D2	27 D2	28 S			21 D2	22 D2	22 D1

Dose cc.	November 10, 1926.				
10^{-9}	17 S				
10^{-8}	20 D3				
10^{-7}	20 D3	19 D2		18 D2	
10^{-6}	22 D2	20 D2		20 D2	
10^{-5}		21 D3		21 D2	
10^{-4}		22 D2		23 D2	
10^{-3}		23 D2		23 D2	
10^{-2}				23 D2	

of pneumococcus, (in a volume of 0.20 cc.) plus 0.20 cc. undiluted serum. Another group of mice would be injected similarly with pneumococcus culture and control rat serum. An additional small group was used to determine the minimum fatal dose of the culture employed. In the dog experiments, some animals were fed the tissues of rabbits killed by pneumococcus, others were fed the living pneumococci. Following the feeding of 275 gm. of tissue, or of the germs from 750 cc. culture per day per dog, for about 2 weeks, the serum was tested in the same manner as the rat serum. There were 4 dogs which were fed the infected rabbit tissues, and 4 controls. These 4 controls were subsequently fed living pneumococci, and compared with 4 new controls, thus making 8 control and 8 experimental dogs. Tests were made for the presence of agglutinins and precipitins, in the sera of the 4 dogs which were fed the infected tissue. Neither agglutinins nor precipitins appear to be present.

Table I shows that the sera of rats, fed tissues of animals killed by pneumococcus possess varying degrees of protective power. The serum of rat 186 protected against 0.01 cc., which was 100,000 times the fatal dose for control mice. Sera of rats 192 and 275, on the other hand, showed no protective action. The others, 187, 189, 191, and 199, were intermediate between these extremes, and uniformly protected against larger doses, than did normal control serum. The sera of immunized rats 189, 191, and 199 were examined for agglutinins and precipitins. The abdominal washings of a mouse killed by pneumococcus injection were used. Neither agglutinins nor precipitins were found.

Table II shows that the sera of dogs fed the tissues of rabbits, killed by injecting pneumococcus, possess protective antibodies in varying amounts. The controls numbered 5, 6, 7, and 8, were subsequently fed live pneumococci and retested. The results are given in Table III. The sera of Dogs 5, 6, 7, and 8 all show an increase in protective power, following the feeding of the tissues of rabbits killed by pneumococcus.

The sera of 3 adult rabbits were examined before and after feeding the living pneumococci from 500 cc. growth per day for 2 weeks. No protective substances were found in their sera.

Following this, experiments were begun to learn whether rats, when immunized by subcutaneous injection of heat-killed pneumococcus, (60° C., ½ hr.) produced protective antibodies with greater regularity than rats immunized by feeding. For this purpose 3 groups of rats were prepared. Each rat in the first group was fed

the living pneumococci from 50 cc. culture per day for 13 days (June 30 to July 23). Each of the second group was fed the acid-killed germs from the same amount of culture for 16 days (June 30 to July 23). Each of the last group received subcutaneously 0.10 cc., 0.20 cc., 0.40 cc., and 0.50 cc. of undiluted heat-killed culture at weekly intervals (on July 2, 9, 17 and 23rd). At different times an animal from each group was sacrificed, its blood centrifuged, and its serum tested for protective substances in the manner described above. Table IV gives the results.

An examination of Table IV shows that there is considerable variation in the quantity of protective antibodies in the sera of rats immunized in a given manner, whether it be by feeding or by subcutaneous injection. It is perhaps possible that the presence of such protective substances is transitory or that they appear in the blood of different animals at different intervals of time following immunization, making detection difficult. Or the findings may be interpreted as further evidence that immunity need not be accompanied by the presence of humoral antibodies.

The results of the rat and dog experiments suggest that a larger animal may be found which, when fed pneumococcus cultures, will produce a serum of therapeutic value. We are now making experiments with this object in view.

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[3] Ross, Victor, *J. Lab. and Clin. Med.*, in press.