CCXXX. A STUDY OF THE FACTORS INFLUENCING THE CONCENTRATION OF COMPLEMENT IN THE BLOOD.

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THE present work was commenced with the idea of determining what changes take place in the development of vitamin A deficiency which renders animals susceptible to bacterial infection. It was felt that this would throw new light on the problem of general immunity, since it presented an easy method of lowering and raising the immunity at will.

Of all known factors in general immunity complement is the one which is most widespread, and, therefore, immediately suggested itself for investigation. It is not possible to say dogmatically that a low blood-complement necessitates a low degree of resistance, as there is not enough evidence to prove this. Much of the evidence up to the present points to this conclusion, however, and many workers in this field seem to assume it [see Cadham, 1926]. Moore [1919] tested this assumption by direct experiment and his results showed that it was true for the animals and conditions he used. Working with a number of guinea-pigs low in complement, he found that, though their specific immunity was not significantly below normal, their general immunity was.

It was decided to determine the complement concentration in the sera from vitamin A-deficient rats at various stages of the deficiency, and to compare this with that of normals. Any differences produced by the administration of cod-liver oil were to be investigated.

Several other problems of a similar nature presented themselves during the course of the work. Indications were found that the age of the animal had an influence, and that the ingestion of food produced a change. Observations on these two points were carried out. Huntermuller [1930] has made the interesting observation that doses of ultra-violet radiation raised the complement in man. A series of observations, therefore, was made on the effect of irradiation by means of a mercury vapour lamp on the blood-complement of rats.

Method of estimating complement.

As it was required to make a large number of estimations on the small amount of blood to be obtained from the tail of a rat any method requiring more than one-tenth of a cc. of serum was excluded. In addition to this, an

attempt was made to avoid the inaccuracy of the "serial tube" methods. After considerable experiment, a modification of Eagle's method [1929] was devised, and the writer has found it to be satisfactory in the course of some three thousand estimations.

Sheep's blood, defibrinated by whipping with glass rods and filtered through muslin soaked in saline, was used as a source of the corpuscles. It could be kept in a cold store for periods not exceeding 3 days. The corpuscles were deposited by centrifuging and were washed 3 times with 20 vols. of normal saline (0.9 % NaCl). They were then shaken with a volume of saline sufficient to make the total volume equal to 10 times the original volume of the blood, thus giving an approximately 5 % suspension of corpuscles, assuming the volume of corpuscles equivalent to about 50 % of original blood. The sensitising agent was glycerinated "anti-sheep corpuscle" serum having 1000 units per cc. (Burroughs and Wellcome).

0.01 cc. of this serum—containing 10 units of complement—was made up to 1 cc. with normal saline, and this was added to 1 cc. of the 5 % suspension of corpuscles.

Before constructing the standard "haemolysis time-complement concentration" curve, it was necessary to measure the complement concentration of a volume of serum by the direct serial tube method. The approximate concentration was determined by adding graded volumes of serum, from 0.0025 to 0.025 cc. in each case made up to 0.05 cc. with saline, to 10 tubes, each containing 0.05 cc. of the suspension of sensitised corpuscles. The tubes were placed in a water-bath at 37° for 15 minutes, and the minimal volume of serum necessary for complete haemolysis was determined. This volume was determined more precisely by repeating the operation, adding volumes of serum covering a smaller range. The liquids were measured with a capillary pipette of 0.05 cc. capacity, graduated into 10 divisions. This was used with a small rubber teat. It was found to be important to empty the pipette at about the same rate each time. The haemolysis mixture was made in agglutination tubes of the Dreyer pattern.

Varying volumes of the serum, whose complement concentration had been determined accurately by the direct serial tube method, were mixed with 0.025 cc. of the 2.5 % suspension of corpuscles in agglutination tubes, and the total volume was made up to 0.2 cc. The time for haemolysis was measured in a thermostat at 37°. The amount of complement used, as calculated from the volume of serum used and the known complement concentration of the serum, was plotted against the time for complete haemolysis (Fig. 1).

In constructing the curve a sample of guinea-pig serum and a sample of human serum were used. In making the estimation on a sample of rat blood, it was the practice to use two or more different volumes of serum, and as the different points fell consistently on the curve, it is clear that the curve is applicable to rat's as well as to human and guinea-pig's sera.

It is the custom to express the concentration of complement as the minimal

volume of serum sufficient to haemolyse a definite volume of corpuscles. A more convenient expression is the reciprocal of this volume. For the purpose of this research, it was decided to adopt, as the unit concentration of complement, that amount which is just sufficient to haemolyse an equal volume of a 2.5 % suspension of sheep's corpuscles, sensitised with 10 units of anti-sheep corpuscle serum, in 15 minutes at 37°. The volume of corpuscle suspension used divided by the minimal volume of serum necessary for complete haemolysis gives the complement concentration of the serum in these empirical units.

Fig. 1. Standard haemolysis time-complement concentration curve.

In order to estimate the complement concentration of an unknown serum, a volume of this serum is mixed with corpuscles as described, the total volume being made up to 0.2 cc. with normal saline. The time for complete haemolysis at 37° is measured. By reference to the curve, the number of units of complement to give this result is ascertained, and, by multiplication, the number of units in 0.025 cc. of serum—the volume of corpuscles used—may be calculated. This figure represents the complement concentration of the serum.

Certain points of technique require special attention. The end-point of complete haemolysis is rather indefinite. To get as good conditions for the observation as possible, a thermostat with glass sides is used, and a brightly illuminated black and white background is placed behind the tubes.

It was found that, in using these small volumes, any visible roughness of the glass was liable to cause errors, probably due to adsorption effects. It is therefore necessary to select very carefully the tubes to be used. Since any deposit left by tap-water is detrimental to the result, the tubes should be rinsed with distilled water several times before being dried. Both in diluting the serum and in adding the corpuscles, thorough mixing is necessary. This can best be done, in small volumes, by blowing one or two small bubbles into the liquid with the pipette and rocking the tubes so that the bubbles act as mixers.

It will be seen from the curve that the points below 120 seconds are rather scattered. Any haemolysis which takes longer than about 240 seconds has an especially vague end-point. Therefore, for accurate work, only the portion of the curve lying between 120 and 240 seconds should be used.

Rous and Turner [1915] advise particular care in the preparation of the washed corpuscles. Their method of drawing the blood into a weak solution of citrate and gelatin may possess advantages, but, as the method of using whipped blood was proving satisfactory, it was not thought advisable to change it. Brooks [1919] used a buffered Ringer solution instead of saline. He used a bicarbonate buffer, which, of course, has the disadvantage of slowly losing carbon dioxide and so becoming more alkaline. It is difficult to see why he chose to work at p_H 7.8, when, if he were going to use a buffer, he might easily have worked at the p_H of the circulating blood.

An idea of the accuracy of the method may be gained from the closeness of the points to the curve in Fig. 1. It has been found that the error is relatively greater in samples of serum poor in complement. This may be due to the relatively greater amounts of serum-proteins present. An analysis of the last 400 duplicate estimations made by the method, using only that part of the standard curve between 120 and 240 seconds, showed the error to be as follows:

	Below 5 units %	5-15 units %	Above 15 units %
Mean deviation ($\frac{1}{2}$ difference of duplicate estimations)	7.6	3.2	2.7
Maximum observed deviation	25	10	7.5

One large source of error was that different samples of sheep's blood differed considerably in the ease with which their corpuscles were haemolysed. This made it necessary to assay each sample. Valley and McAlpine [1928] state that a good way to preserve complement in serum is to keep it away from corpuscles in an atmosphere of carbon dioxide. By preparing a standard sample of serum and keeping it under these conditions, and by titrating each sample of corpuscles against it, a curve for the depreciation of complement in this serum was obtained. A correction was applied to each of those samples of serum which showed a deviation from the curve.

To obtain a sample of blood from the rat, the tail was warmed in normal saline at 45° for 1 minute; the end was cut with a pair of scissors and a few drops were squeezed out on to a waxed plate. A piece of glass tubing had one end drawn out into a capillary. The blood was drawn up into the tube through the capillary portion before it had time to clot, and the end of the capillary was sealed in a flame. When a clot had formed, a fine glass needle was passed between it and the walls of the tube to prevent the clot sticking to the glass. The tube was stoppered and allowed to stand overnight. No appreciable change took place if the sample was kept in a cold store for 48 hours.

INFLUENCE OF DIETARY DEFICIENCY.

Early experiment. Two groups of animals which were at hand offered an opportunity of testing whether animals on a poor basal diet differed from animals which had taken a liberal supplement of vitamin-containing foods. Rats 420 to 426 had been fed on mixed corn and brown bread from the time of weaning. This is a poor diet, though it is not absolutely deficient in any vitamin. Rats 325 to 328 had received a diet of purified caseinogen, starch and salts, with 3 % cod-liver oil. During part of this time they had been deprived of one of the constituents of the vitamin B complex, but, before the time of the commencement of the experiment, they had been brought to a normal condition by the addition of 6 % marmite to their diet. These animals were put on a diet of corn and brown bread. Another litter of rats was treated in a similar manner. Four, A 18 to A 21, were given corn and brown bread alone, and two, A 17 and A 22, had a liberal supply of green stuff, milk and cod-liver oil added to this diet.

All the animals were given 1 drop of "radiostol" per week throughout the experiment to ensure a good supply of vitamin D. According to the specification of the makers, this is equivalent to about 40 rat units per day, so that even allowing for some depreciation the animals should have been taking an excess of vitamin D.

Each sample was taken in the morning after a fast of 16 hours. The results are given in Table I. It will be seen that the animals which had received a rich

Table I.

A, 3 days after 10 drops of cod-liver oil (0.33 cc.). B, 4 days after 10 drops of cod-liver oil.

Basal diet <i>plus</i> excess cod-liver oil, milk and greens		Basal diet. Mixed corn and brown bread	
Rat No.	Complement	Rat No.	Complement
325	15.0	420	4.6
	12.7		6.1
	14.0	421	1.9
	13.0		6.4
326	12.2	422	1.8
	17.1		10.3 A
	14.7	423	4.7
	10.7		8.7
327	10.7		15.6 B
	16.9	424	10.7
328	13.5	425	6.4
	15.4	426	6.6
A 17	7.3	A 18	3.4
	20.8		3.1
A 22	9.0	A 19	3.4
	16.8		3.0
		A 20	3.1
			3.1
		A 21	8.6
			6.2

diet had much more complement than the others. It was significant that rats 422 and 423 had a high complement a few days after a dose of 10 drops of cod-liver oil.

The basal diet seemed to have no absolute deficiency, and it was not clear what it was in the supplements which caused the difference in complement concentrations. It was decided to perform an experiment to compare complement in animals fed on a diet deficient in vitamin A with that in others having cod-liver oil added to the diet. Before this was done it was considered to be advisable to investigate in more detail the influence of age, the ingestion of food and illumination.

The effect of the age of the animal.

In the course of previous investigations indications were found that there was a tendency towards a low complement in young rats. This does not fit in with the findings of workers on human beings [see Goldner, 1929; Gunn, 1914; Moro, 1902]. Kolmer, Matsunani and Trist [1919] state that the blood of young guinea-pigs is more frequently low in complement than that of adults, but give no figures in support of this statement.

Complement titrations have been performed on blood from 43 young animals between the age of weaning and 4 weeks from weaning, the animals being weaned between the ages of 3 and 5 weeks according to their weight. Almost all the samples were found to be poor in complement as compared with normal adult animals, whether the samples were taken in the morning after a 16-hour fast, or in the afternoon 3 hours after feeding (Table II and the distribution curves of Fig. 2). In making the comparison, only those figures for young rats are used which are directly comparable with the adult normals, *i.e.* only estimations on blood samples taken in the morning after 16 hours of fasting. The young rats include animals both on a deficient diet and on a rich diet.

The results show that there is a marked tendency for the complement to be low in the blood of young rats and, therefore, only rats of more than 4 weeks from weaning should be used in these experiments.

The effect of ingestion of food.

In the course of the main investigation, indications were received that, in some cases, the ingestion of food caused a change in complement. Cadham [1926], Hadjapoulos and Burbank [1928] and Gunn [1914] mention that a slight rise in complement may be noticed during the day, but they do not correlate this with any digestive activity. Kolmer, Matsunani and Trist [1919]

Fig. 2. Percentage distribution of complement values in young and adult rats.

○—○ Young rats.
●—● Normal adult rats.

Table II. *Comparison of complement concentrations in the blood of young and adult rats.*

	Young rats		Normal adult rats	
	No. in range	% in range	No. in range	% in range
0- 2 units	1	2	0	0
2- 4 "	3	8	0	0
4- 6 "	8	20	2	3
6- 8 "	13	33	0	0
8-10 "	10	25	4	7
10-12 "	1	2	14	24
12-14 "	3	8	18	31
14-16 "	0	0	16	29
16-18 "	1	2	2	3
18-20 "	0	0	2	3

	Young rats	Normal adult rats
Percentage below 2 units	2	0
" " 4 "	10	0
" " 6 "	30	3
" " 8 "	63	3
" " 10 "	88	10
" " 12 "	90	34
" " 14 "	98	65
" " 16 "	98	94
" " 18 "	100	97
" " 20 "	100	100

state that blood taken after a 24-hour fast is richer in complement than blood taken immediately after a meal.

A short investigation was made to ascertain whether any definite changes occurred. Out of 23 experiments, 11 showed no change, 12 showed definite changes, comprising a definite fall, a rise and then a fall, or a steady rise (Table III). The basal diet referred to is the fat-free vitamin A-deficient diet described later.

Ehrlich [1906] has suggested that the mechanism for the destruction of bacteria in the blood is the same as that for the utilisation of food material carried by the blood. On this hypothesis, it would be expected that, under certain conditions, a change in some of the agents of immunity might be found during digestion of food. This change might also be expected from a consideration of the suggestion of Walker [1905-06] that the enzymes themselves use a "complement."

Sufficient data have been obtained to show that in some circumstances and in certain animals a change does take place. What the conditions are under which a change occurs, and what regulates the type of change, are problems which will be investigated at a future date.

Effects of irradiation with a mercury vapour lamp.

Huntermuller [1930] investigated the effects of ultra-violet light in winter on 18 human beings, most of whom were normal. His conclusion is that a good, but not excessive, dose causes an immediate slight rise in complement, then a

Table III. *Changes in complement concentration after ingestion of food.*

Rat 289. Basal diet plus 10 drops olive oil			Food intake g.	Rat 432. Basal diet			Food intake g.	Remarks
Initial	16.7	—		Initial	16.2	—		
1 hour	16.7	6		1 hour	15.6	8		No change outside limits of accuracy of the method. Eleven results of this type
2 hours	15.4	5		2 hours	15.8	0		
3 hours	15.4	1		3 hours	15.4	0		
4 hours	15.4	2		Total food...		8		
5 hours	16.7	1						
Total food...			15					
Rat 369. Basal diet				Rat 331. Basal diet				
Initial	11.2	—		Initial	13.6	—		Definite immediate fall. Five results of this type
1 hour	10.0	2		1 hour	10.0	3		
2 hours	9.2	2		2 hours	10.6	2		
3½ hours	10.2	2		3 hours	11.6	1		
5 hours	8.3	1		4 hours	12.0	1		
Total food...			7	5 hours	14.3	0		
				Total food...		7		
Rat 432. Basal diet				Rat 423. Basal diet				
Initial	8.7	—		Initial	4.8	—		Rise followed by a fall. Four results of this type
½ hour	9.7	10		1 hour	13.0	10		
1½ hours	6.9	0		2 hours	5.6	2		
3 hours	6.0	2		3 hours	5.3	0		
Total food...			12	5 hours	5.3	0		
				Total food...		12		
Rat 327. Basal diet				Rat 332. Basal diet				
Initial	14.0	—		Initial	4.0	—		Steady rise. Three results of this type
1 hour	15.4	6		1 hour	4.0	10		
2½ hours	17.0	0		2 hours	11.0	0		
4 hours	19.0	0		3 hours	11.3	3		
Total food...			6	4 hours	11.5	2		
				5 hours	13.5	0		
				Total food...		15		

fall, and, in 2 or 3 hours, a steady rise reaching a maximum in about 20 hours, then a gradual fall back to the initial value in the course of several days. Unfortunately he quotes no figures to show the extent of the rise, and he does not state whether he finds that it occurs invariably or only in a majority of cases.

In investigating the effects of irradiation on the complement, a mercury vapour lamp was used which produced a definite erythema on human skin at a distance of 6 inches in 2 minutes. The animals were placed in their cages, all food having been removed, at a distance of 20 inches from the lamp. Since the cages had a metal grid bottom, there was no possibility of an effect from the ingestion of irradiated sawdust from the tray of the cage. The experimental animals were controlled by animals, if not from the same litter, of the same age and having an identical feeding history. The controls were kept in cages

apart from the experimental animals, but they were kept under the same conditions during the experiment, being carried into the lamp room with the experimental animals to control any effect of handling or of temperature change. In some cases, a control experiment was performed on the same animal, either before or subsequent to the experimental observation.

Twenty-eight animals were used in this work. Twenty-six experimental and 18 control observations were carried out. In most cases an observation consisted of taking a sample at 10 a.m., immediately before the irradiation, another at 12.30 p.m., and a third at 6 p.m. In a few experiments longer intervals were allowed between sampling. The exposure varied from 2 to 4 minutes at 20 inches.

A typical example of the results obtained is given in Table IV. Of the 26 observations, 6 gave a definite rise, 3 gave a doubtful rise and 17 gave no significant rise above the controls. The important fact, from the point of view of the main thesis of this work, is, that none of the 10 rats on the vitamin A-deficient diet submitted to the light showed a significant rise in complement. The data therefore show that, while the mercury vapour lamp causes a rise in complement in rats on some occasions—a fact which corresponds with Huntermuller's

Table IV. *Examples of the effect of irradiation with a mercury vapour lamp on complement.*

		Complement			Remarks
		10 a.m.	12.30 p.m.	6 p.m.	
Rat A 17	3 mins. irradiation	3.2	8.1	10.9	Rise
Rat A 22	Control	3.8	3.2	4.0	
Rat A 18	3 mins. irradiation	1.8	3.3	3.5	No rise
Rat A 19	„ „	1.9	2.8	5.4	? rise
Rat A 20	Control	1.7	1.7	2.9	
Rat A 21	„ „	1.7	2.3	3.3	
Rat 381	3 mins. irradiation	7.8	10.0	13.1	Rise
Rat 420	„ „	14.5	11.4	16.3	No rise
Rat 479	„ „	7.8	5.8	10.3	No rise
Rat 421	„ „	11.0	9.0	13.4	

observations on human beings—it does not do so invariably. This conclusion is further strengthened by the observation that 8 vitamin A-deficient rats and 7 young rats, which were daily exposed to the radiation for 2 to 3 minutes, all had a complement figure below 8 units on at least one occasion.

Effect of a diet deficient in vitamin A.

The main experiments which follow were controlled by rigidly defining the ages of the animals and the time of estimation of the complement after feeding. Further, the animals were all kept in a darkened room. No rat was used in this experiment under 4 weeks from weaning.

Twenty-four animals from 5 litters were put on a diet of:

	g.
Purified rice starch	70
"Glaxo" vitamin A-free caseinogen	20
McCullum's salt mixture	5
Agar	2
Marmite	6

The marmite was increased to 10 g. during the course of the experiment, as on theoretical grounds 6 % is barely sufficient for the maintenance of an adequate supply of the vitamin B complex. Some of the animals, as controls, were given a supply of the fat-soluble vitamins either as cod-liver oil or as "radiostoleum." All the others were given 1 drop "radiostol" per week to ensure a supply of vitamin D.

As the absence of fats from the diet might have had an effect on the complement level, another group of animals was given

	g.
Rice starch	50
Hardened cotton-seed oil	20
McCullum's salt mixture	5
"Glaxo" vitamin A-free caseinogen	20
Marmite	10

As before, some of these animals were kept as controls and were given 5 drops of cod-liver oil per day, and the others were given 1 drop of "radiostol" per week.

Blood samples were taken from these rats at intervals of about a week. In the beginning and during most of the course of the experiment, the samples were drawn in the morning, after a 16-hour fast. During the latter part of the experiment a few samples were taken at 3.30 p.m., 3 hours after new food had been put in the cages.

In all, 36 animals were used on this part of the work. Twenty-four were placed on the vitamin A-deficient diet, and 12 were given an adequate supply of the vitamin in the form of cod-liver oil or "radiostoleum," and showed no abnormalities in growth or symptoms associated with vitamin A deficiency. An analysis of the figures obtained showed that, while the complement only fell below 10 units in 2 out of 12 normal, it fell below that level in 22 out of the 24 vitamin A-deficient animals.

A statistical analysis of the figures is shown graphically in Fig. 3, and in tabular form in Table V. Each point in the first figure represents a reading between the nearest whole numbers. In the second figure the percentages of the total number of readings within certain ranges are plotted for normal and deficient animals. Only readings obtained after a 16-hour fast have been reckoned; those taken at 3 hours after feeding gave a similar result.

Fig. 3. Percentage distribution of complement values.
 ●—● Normal rats.
 ○—○ Deficient rats.

Table V. *Distribution of complement estimations for rats on basal diet deficient in vitamin A and the same supplemented with cod-liver oil.*

	Rats on basal diet		Rats on basal diet <i>plus</i> cod-liver oil	
	No. in range	% in range	No. in range	% in range
0- 2 units	3	4	0	0
2- 4 "	3	4	0	0
4- 6 "	11	14	2	3
6- 8 "	12	15	0	0
8-10 "	18	22	4	7
10-12 "	14	17	14	24
12-14 "	10	12	18	31
14-16 "	8	10	16	29
16-18 "	1	1	2	3
18-20 "	1	1	2	3

Percentage below 2 units			Rats on basal diet	Rats on basal diet <i>plus</i> cod- liver oil
	4		4	0
"	" 6 "		8	0
"	" 8 "		22	3
"	" 10 "		37	3
"	" 12 "		59	10
"	" 14 "		76	34
"	" 16 "		88	65
"	" 18 "		98	94
"	" 20 "		99	97
"	" 20 "		100	100

It may be concluded, from the results obtained that there is a marked tendency towards low blood-complement in animals deficient in vitamin A or some other factor, other than vitamin D, occurring in liver oils. In the deficient animals marked fluctuations in complement were found which may be due, in part, to the infections to which such deficient animals are susceptible, and, in part, to whatever it is that causes fluctuations from day to day in normal rats and human beings. The fact that animals which are well supplied with liver oil are occasionally deficient in complement, indicates that the presence of liver oils in the diet is not the only condition which must be fulfilled for the maintenance of a high complement. It has been a common experience in bacteriological laboratories that the complement of guinea-pig's serum, high in the summer, may be low in the winter without any alteration in the diet. It is significant that the few instances in which a low blood-complement has been found in rats receiving cod-liver oil have all occurred in winter months.

DISCUSSION.

Irregularity of results. One of the most noticeable features of the results recorded in this work, and, indeed, of any other work which has been done involving the determination of haemolytic complement, is the irregularity of the results. This must be due to the existence of factors influencing complement which have not yet been discovered. This variability makes it necessary

to obtain a large number of observations, and to describe any changes as "tendencies" instead of as definite changes.

In considering these results it should be borne in mind that weight curves of rats deprived of vitamin A are seldom even, and that there is very little constancy about the reaction of deficient animals to cod-liver oil. The day-to-day variation of complement in human beings and rats is difficult to explain. Some of the reported variations have undoubtedly been experimental artefacts due to a variation in the corpuscles used in the haemolysis system. Even when a correction is made for this by the method described for assaying the susceptibility of corpuscles, there is still a large variation.

Relation to other deficiencies. Although lowered resistance is not as prominent a symptom of vitamin B, C, or D deficiency as it is of vitamin A deficiency, there is good support for the view that it is found in these conditions. Werkman, Nelson and Fulmer [1924] showed that guinea-pigs suffering from scurvy were less resistant to bacterial infection than normals, and Findlay [1925] confirmed this. Werkman [1923] showed that rats and pigeons lacking vitamin B were less immune to attacks from *B. anthracis* and the *Pneumococcus* than normal animals. Findlay and Maclean [1925] found that blood from animals deficient in vitamin B or vitamin D had a lowered bactericidal power. Smith and Wason [1923] confirmed this for vitamin D-deficient animals.

The question of whether the lowering of complement in animals fed on a diet deficient in vitamin A is specific, or whether it is common to all vitamin deficiencies, has had some attention. In a cursory investigation, consisting of 8 observations on the blood of 4 rats deficient in vitamin B₁, the author found no significant change in complement. This, however, should by no means be accepted as conclusive. Zilva [1919] found no change in guinea-pigs on a scorbutic diet. Koch and Smith [1924], on the other hand, found a slight rise during the development of scurvy. Smith and Wason [1923] found no change in rats fed on a rachitic diet.

Inanition due to uncomplicated starvation is generally thought to lower resistance to bacterial infection. Koch and Smith [1924] found a drop in complementary power during starvation, but Zilva [1919] found no loss of complement in guinea-pigs after they had been kept on a quantitatively restricted diet for 6 months.

It is certain that there is a lowered resistance in dietary disturbances other than vitamin A deficiency. The evidence is against these being due to diminished complement activity, but this should not be considered as proven.

The action of ultra-violet radiation. The action of ultra-violet light on the body is a much disputed subject. Many people have claimed that it raises the resistance of the body to infection. Colebrook, Eidenow and Hill [1924] showed that it raised the bactericidal power of the blood. This increase was of such a transitory nature that many people were unwilling to attach much importance to it. Colebrook [1929] undertook an extended research and failed to find that ultra-violet light improved the general health in any way, while Hill and Lawrie

[1931] reaffirmed the claim of many that it did. The increased resistance produced by ultra-violet light is thought to be quite apart from its well-known power of producing vitamin D.

The finding of Huntermuller, that ultra-violet light increases complement, is a big advance and may be the means by which many of the actions of ultra-violet light will be explained. The finding that this increase does not occur in all cases of laboratory animals does not detract from the importance of Huntermuller's discovery. These exceptions are of interest in this research because they make it unlikely that ultra-violet light and cod-liver oil cause a rise in complement through the same agency. The evidence for this is that none of the vitamin A-deficient animals submitted to ultra-violet light reacted with a definite rise in complement, and a number of animals which were daily exposed to irradiation had low complements during periods of vitamin A deficiency.

SUMMARY.

1. A method of estimating haemolytic complement is described, which has greater accuracy and uses smaller quantities of blood than the serial tube method now in general use.

2. A unit of complement is defined, which is the reciprocal of the minimal volume of serum sufficient to haemolyse a given volume of sensitised blood corpuscle suspension.

3. Young weaned rats tend to have a lower complement than adult animals.

4. The ingestion of food may cause a variation in the blood-complement of rats.

5. Irradiation with a mercury vapour lamp sometimes causes a rise in complement (Huntermuller), but enough evidence has been obtained to show that this effect is not general.

6. The haemolytic complement tends to be lower in the blood of rats fed on a diet deficient in vitamin A than in the blood of controls receiving cod-liver oil.

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