

occur nor does contraction commonly set in as a later event. In the usual form of glomerulo-nephritis, on the other hand, the degree of capillary blockage is much greater, leading to functionless hyaline glomeruli, atrophied tubules, contraction of the kidneys, and to uraemia. Lipoid nephrosis does not terminate in uraemia. In cases which do not recover, death is most commonly due to peritonitis, pneumonia, or septicaemia. The frequency of peritonitis is remarkable, as it accounts for almost half the deaths; where the responsible organism has been isolated it has been found to be pneumococcus.

There can be little doubt that the lipid which is deposited in the renal cells, and which finds its way thence into the urine, is an outcome of the very high cholesterol content of the blood. Its constant deposition in the convoluted tubules, and to a lesser extent in the glomerular cells, suggests that an endeavour is being made to excrete the surplus. As to the cause of the cholesterolaemia we have no certain knowledge; it may be secondary to the great loss of albumin in the urine sustained by sufferers from this form of renal disease. As in diabetes, so in lipoid nephrosis, mobilization of fat from the reserves may occur in an attempt on the part of the system to feed tissues partially starved by the constant drainage from the body, in the one case of carbohydrate, in the other of protein. Of the fat so mobilized, the cholesterol fraction is stable and relatively valueless as a source of energy. It seems possible that this fraction is taken up by the renal cells, and in particular by the cells of the convoluted tubules, where it is deposited, some eventually passing away in the urine either by disruption or shedding of the cells. This process of excretion of cholesterol is analogous to a physiological process by which an isomer of cholesterol

is excreted in the normal individual: the secretion of the sebaceous gland involves a degenerative process in which isocholesterol is first laid down in the gland cells, and later, either by disruption of the cells or by their separation from the gland, is discharged and lubricates the skin and its appendages.

Finally, as to the cause of lipoid nephrosis: in view of the probability that it is in reality a chronic mild glomerulitis, one should bear in mind that two bacterial toxins are definitely known to select the glomerulus of the kidney for attack. They are the toxins of the diphtheria bacillus and of the haemolytic streptococcus. The cause of lipoid nephrosis is unknown, but there is justification for suspecting a bacterial origin. One organism, the pneumococcus, suggests itself for study. Why is it that so many of the patients die of pneumococcal peritonitis?

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## INSOLUBLE PRECIPITATES IN DIPHTHERIA AND TETANUS IMMUNIZATION.

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THE addition of aluminium sulphate or potash alum to a diphtheria toxoid produces a relatively insoluble precipitate which has high antigenic properties. Glenny, Pope, Waddington, and Wallace (1926), and Glenny and Waddington (1928), have shown that such precipitated toxoid immunizes guinea-pigs more readily than the original toxoid.

In view of the promising results obtained,\* it is possible that the most favoured method of human immunization against diphtheria in the near future may be by means of a suspension of toxoid precipitated by alum; it appears to be of interest, therefore, to present some results of animal experiments with both diphtheria and tetanus toxoid showing the extent to which the antigenic efficiency is increased by the addition of alum. During the course of our experimental work different quantities of alum from 0.1 to 2 per cent. have been added; in some instances the whole mixture of toxoid and alum has been used; sometimes the precipitate has been separated from the supernatant liquid and re-suspended after washing in saline. It is not within the scope of this paper to deal with these differences.

The immunity index method of Glenny, Allen, and Hopkins (1923), more fully described by Glenny and Waddington (1928), forms a convenient method of testing antigenic efficiency. Table I shows the immunity index given by the injection into guinea-pigs of 0.1 c.cm. of diphtheria toxoid with and without varying amounts of alum. It will be seen that when alum had been added to the toxoid, 29 guinea-pigs out of 41 were Schick-negative when tested three weeks later, and all were immune by the fifth week. Without alum none, out of 38, was immune after three weeks, and 8 were still Schick-positive seven weeks after injection.

\* Since this paper was written, W. H. Park, at the Paris Congress of the International Society of Microbiology, reported 100 per cent. successful immunizations with alum-toxoid in small groups of children.

A further series of guinea-pigs was injected with 1 c.cm. of diphtheria toxoid, and the antitoxic titre of serum was tested four to six weeks later. Of 53 guinea-

TABLE I.—Showing the immunity index of guinea-pigs injected with 0.1 c.cm. diphtheria toxoid with and without alum.

	Number of guinea-pigs giving an index of					
	1	2	3	4	5	Over 5.
With alum ...	29	10	2	—	—	—
Without alum ...	0	10	12	5	3	8

pigs injected without alum, the majority contained 0.02 unit per c.cm. of serum, and the highest value recorded was 0.5 unit; of 23 injected with alum the majority contained 0.5 to 1 unit, and the highest recorded value was 7 units per c.cm. The results of the group are summarized in Table II.

Alum has also been added to diphtheria toxin used in the hyperimmunization of horses. The average antitoxic

TABLE II.—Showing the antitoxic value of the serum of guinea-pigs four to six weeks after the injection of 1 c.cm. diphtheria toxoid with and without alum.

	No. of guinea-pigs with an antitoxic value of (per c.cm.)				
	Under 0.001 unit.	0.0-0.01 unit.	0.01-0.1 unit.	0.1-1 unit.	Over 1 unit.
With alum ...	—	—	1	9	13
Without alum	3	6	31	13	0

value of groups of 50 horses was 696 without alum; with alum 1,102.

Many experiments have also been made with tetanus toxoid and alum. Table III gives the results of an experiment on guinea-pigs injected with 0.1 c.cm. tetanus toxoid diluted to 5 c.cm. with saline, or with 0.01 or 0.1 per cent. potash alum. The injections were repeated four weeks later, and the guinea-pigs were injected with tetanus toxin two to three weeks after the second

injection, as a test for the immunity produced. It will be seen that only 3 out of 13 guinea-pigs receiving toxoid without alum survived 1/50 of a test dose of toxin, and out of 4 none survived 1/10 of a test dose. Of the guinea-

TABLE III.—Showing the number of guinea-pigs surviving different doses of tetanus toxin given two to three weeks after the second of two doses (given at four weeks' interval) of 0.1 c.cm. tetanus toxoid diluted to 5 c.cm. with saline or with alum.

Toxin in terms of test doses.	Number of survivals out of total tested.		
	Diluent Saline.	0.01% alum.	0.1% alum.
0.01	2 out of 4	—	—
0.02	3 out of 13	2 out of 2	2 out of 2
0.05	—	1 out of 2	2 out of 2
0.1	0 out of 4	3 out of 4	2 out of 2
0.2	—	—	2 out of 2
1.0	—	2 out of 2	2 out of 3
10.0	—	2 out of 2	2 out of 2
100.0 (over 20,000 M.L.D.)	—	0 out of 1	4 out of 4

pigs given alum-toxoid, nearly all survived the injection of toxin, and 4 out of 4 survived 100 test doses.

Tetanus alum-toxoid has proved of great service in the immunization against tetanus of horses used for the production of therapeutic serum. The majority of horses given one injection of toxoid with alum have over 1 unit (U.S.A.) per c.cm. of serum four weeks later. Those given toxoid without alum rarely have over 0.001 unit. A comparison between the results with and without alum is given in Table IV. In a further series of 17 horses

TABLE IV.—Showing the antitoxic value of horses four weeks after a single injection with 10 or 50 c.cm. tetanus toxoid with varying doses of alum.

Toxoid.	Percentage of Alum.	Number of horses with antitoxic value of (per c.cm.)			
		0.0002 unit.	0.0002-0.01 unit.	0.01-1 unit.	1-30 units.
50 c.cm.	0.5-2.0	0	1	8	23
10 c.cm.	0.5-2.0	0	1	5	4
50 c.cm.	0-0.1	8	4	0	0
10 c.cm.	0-0.1	2	0	0	0

receiving toxoid without alum, 1 had 0.01 unit, and the others less than this amount; of 156 horses with alum, 124 gave over 1 unit; of these 86 had over 3 units, 20 over 10 units, and 1 had 25 units per c.cm. of serum as a result of a single injection. All the horses were tested before injection, and were proved to contain no normal tetanus antitoxin.

In the hyperimmunization of horses the addition of alum to tetanus toxin has doubled the value of the antitoxin produced. Glenny, Pope, Waddington, and Wallace (1925) showed that if horses were rested for a month or more after a primary stimulus before tetanus immunization was continued, much stronger antitoxin was produced. Ramon and Descombey (1930) have recently confirmed this. Horses with a primary stimulus of alum-toxoid that have been rested at least a month before the commencement of their main tetanus immunization produced an average of 800 (U.S.A.) units per c.cm.

A somewhat similar but smaller effect than that produced by alum has been obtained by Ramon (1926) by the addition of tapioca to toxoid. Ramon, observing that horses developing abscesses at the site of injection yielded serum of higher antitoxic value, tried various substances that would produce sterile abscesses, and eventually chose tapioca as the most suitable. It appears probable that in both cases increased antigenic response is due to delayed absorption. Alum was first tried by us in 1925,

when we wished to determine the effect of delaying absorption by presenting toxoid in a relatively insoluble form. My colleague Mr. Pope suggested the alum precipitate as a suitable product. Recent work has shown that the original idea is probably correct. Dr. Buttler, continuing this work, has found that several substances which produce relatively insoluble precipitates of toxoid also increase antigenic efficiency. Other work, not yet published, has established that toxoid, injected as a primary stimulus, is excreted with extreme rapidity, and that within a few days of injection not 1 per cent. remains in the animal body. The increase in efficiency of alum toxoid is therefore probably caused by its slow rate of absorption, so that antigenic stimulation is maintained for many days. The addition of alum to toxin has also been used with success in the immunization of horses against the toxins of scarlet fever, *B. welchii*, and dysentery.

Summary.

The immunity produced by the precipitate, formed by the addition of alum to tetanus and diphtheria toxoids, is greater than that produced by toxoid alone. The increase in certain experimental conditions has reached even a thousandfold.

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TETANY AFTER EXERCISE:

A CLINICAL AND CHEMICAL STUDY OF A CASE.

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TETANY after exercise is extremely rare. In a series of clinical cases of tetany due to overbreathing Goldman<sup>1</sup> refers to some students who developed tetany after a fight, but they were not personally observed by him. The only other example in the literature is that recorded by Collier<sup>2</sup>; the underlying cause in this case was never investigated. Recently we have had the opportunity of studying such a case in detail, and have clearly established the etiology of the tetany, which has proved to be alkalaemia following functional overbreathing.

*History.*—The patient, a girl aged 16 years and 9 months, was referred to us for examination by Dr. C. Attenborough of Camberley. Tetany first came on two and a half years ago after a lacrosse match at school; since then she had shown definite signs of tetany about six times at irregular intervals. The attacks began, as a rule, about ten minutes after games, such as tennis or lacrosse, and lasted half an hour. She never suffered during the holidays, even after vigorous exercise. Quite recently she had three seizures following the sudden death of her head mistress, which was a great mental shock. Once lately she had tetany after quite a gentle walk, and once actually in bed about noon, half an hour after Dr. Attenborough had called to see her. Calcium lactate (5 grains) and parathyroid (1/40 grain) had been given by the mouth by Dr. Attenborough, who thought that this had once been beneficial, but was recently much less so. The girl's general health has always been satisfactory, and she is described at school as "clever, good at games, and very highly strung."

Previous history disclosed nothing of etiological importance. She was breast-fed for two months, and then given Savory and Moore's food. Inquiry and physical examination revealed no evidence of rickets. Her only illnesses were chicken-pox and German measles. Menstruation began at the age of 13½, and has been regular. There have never been any of the gastro-intestinal disturbances which are associated with some types of tetany.

The family history contained nothing of importance. An elder brother had had asthma. Two younger brothers were healthy.