bile capillaries or in that zone of the cell which immediately surrounds the capillaries.

In the kidneys of these animals the fat first appears in the loops of Henle. The fat is most pronounced in the ascending limb of Henle's loop.

In puppies and young dogs the fat is in small amount and appears in the form of minute granules in the epithelium lining these tubules. In adult animals and especially in old animals the fat is very greatly increased in amount and is seen in the form of large granules which may coalesce to form masses which serve to outline the course of the tubule.

There is apparently an association between the amount of fat found in the liver and kidney of animals of different ages with the amount of glucose present in the urine. The puppies and young animals which show a low percentage of glucose in the urine show a small amount of fat in the liver and kidney.

The full-grown animals and old animals which have shown an earlier appearance of glucose in the urine and a percentage of glucose which has been much higher than has been found in the puppies also show fatty changes in the liver and kidney of much greater severity.

100 (917)

Variations in resistance of red blood cells in sheep.

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In the course of complement fixation work we have noticed that the specimens of blood cells obtained from different sheep under exactly similar conditions occasionally show marked differences in their susceptibility to laking by specific lytic serum. When this was first noticed we were using two sheep as a source of blood, and as the sheep whose cells were more highly resistant was one which had been bled repeatedly and profusely it was natural to attribute the increased resistance of the cells to this.

We determined to investigate this question and also to find out whether the increased resistance was a specific resistance to

laking by amboceptor and complement or was a general resistance such as would be shown by an increased resistance to laking by hypotonic salt solution, and also whether the sera of different sheep show similar differences in their tonicity.

Three sheep were used:—

T—an apparently normal young male sheep, newly obtained from the dealer.

F—a sheep which had been kept in the laboratory for six months and had been bled repeatedly and on occasions profusely.

P—a sheep which had been confined for two and one half years, had been bled occasionally and had received immunizing injections of typhoid bacilli for a period of about eighteen months.

F was slightly and *P* decidedly anemic. The blood of all three was obtained on the same day and the cells were washed with great care to handle the blood of all three animals in precisely similar ways. All three were made up into 5 per cent suspension and tested with diminishing quantities of hemolytic amboceptor (rabbit serum) and a fixed dose of 10 per cent complement (guinea pig) with the following result:

TABLE I.

VARIATIONS IN RESISTANCE TO LYTIC SERUM.

Each tube contains 0.5 c.c. 5 per cent. washed red cells, 0.1 c.c. of 10 per cent. guinea-pig complement and the amount of immune serum indicated below. Incubation one-half hour.

(1/600) Amboceptor.	.1	.15	.2	2.5	3.0
Cells of sheep *P*.....	+++	++++	++++	++++	++++
Cells of sheep *F*.....	++	+++	++++	++++	++++
Cells of sheep *T*.....	+	++	++++	++++	++++

(++++ = complete hemolysis.)

It is seen that the new sheep, *T*, was the most resistant to laking, the frequently bled sheep, *F*, almost as resistant, and the typhoid injected sheep, *P*, least resistant.

At the same time the washed cells were tested for their susceptibility to laking by anisotonic salt solutions, by adding .2 c.c. of 30 per cent suspension of cells of each sheep to 5 c.c. of salt solution whose strength ranged from .3 per cent to 9 per cent with intervals of .025 per cent.

The new sheep (*T*) was then bled 800 c.c. and the blood of all

three animals again tested on the first, second and sixth day after this bleeding.

For the sake of brevity the complete data obtained with the salt solutions are not given but only the point of beginning hemolysis and of complete hemolysis. They are as follows:

TABLE II.

Date.	Hemolysis begins.	Hemolysis complete.
	Sheep T.	
April 22	.600	.400
April 23	.575	.400
April 24	(.525)	.400
April 28	.575	.400
	Sheep F.	
April 22	.600	.450
April 23	.575	.450
April 24	(.525)	.450
April 28	.575	.450
	Sheep P.	
April 22	.700	.525
April 23	.675	.500
April 24	( )	
April 28	.650	.525

It is seen that hemorrhage had practically no effect on sheep *T* (the variations are within the range of experimental error). The resistance of each animal was about the same on each day.

The cells on each day were also again tested with amboceptor and complement and without giving charts of the results it may be stated that the differences between the three sheep were substantially the same as they were before the bleeding of sheep *T* on each of the four days.

It is interesting to note that the resistance to immune serum on each day ran parallel to the resistance to hypotonic salt solution, the cells of sheep *P* always being most easily laked, those of sheep *F* next and those of sheep *T* least easily laked.

We also attempted to determine whether the differences in susceptibility to laking by immune serum were due to differences in ability of the different sheep's cells to absorb amboceptor. This was found not to be the case: at least the cells of all three sheep were able to absorb a great excess (at least 30 units) of

amboceptor, and likewise the titrations of the different cells against immune serum gave the same relative results whether the amount of immune serum was kept constant and the amount of complement varied, or the amount of complement was kept constant and the amount of immune serum varied.

The serum of the three animals was also tested for its tonicity by diluting with graded amounts of distilled water and adding to 2 c.c. of each dilution .1 c.c. of 30 per cent suspension of freshly washed cells from sheep *F*. The serum of sheep *T* did show a very slight lowering of its tonicity following the hemorrhage. The sera obtained on three different days were tested simultaneously.

TABLE III.

HEMOLYSIS OF RED CELLS OF SHEEP *F* IN SERUM OF *T* DILUTED WITH DISTILLED WATER.

Per Cent. of Serum.	50	52.5	55	57.5	60
Serum of *T*, April 22	++	+	+	−	−
Serum of *T*, April 23	+++	++	+	+	−
Serum of *T*, April 24	+++	++	+	+	−

(++++ = complete hemolysis.)

The tonicities of the sera of the three animals were very nearly the same and had no relation to the rather wide variations in the resistance of their red cells.

Smith and Brown working with horses' blood found marked variations from the average resistance to salt solutions of low tonicity. About 10 per cent of horses have red cells very sensitive to hypotonic solutions. The sera of these horses were rather constant in tonicity. Cornwall[1] found that the apparent tonicity of sheep serum has no relation to the mean lytic point of the red blood corpuscles of the individual and is largely due to lipoids. He also found marked variations in individual animals.

Experiments of different workers on the effect of hemorrhage on the resistance of blood cells have given varying results. Smith and Brown[2] working with horses found a slight decrease in the resistance to hypotonic salt solution only after many large hemorrhages and only in some individuals. Itami and Pratt[3] also working with rabbits, found a slight increase in resistance.

[1] Cornwall, *Jour. of Hygiene*, Oct., 1912, Vol. 12, p. 245.

[2] Smith and Brown, *Jour. Med. Res.*, 1906, Vol. 15, p. 415.

[3] Itami and Pratt, *Biochem. Zeit.*, 1909, Vol. 18, p. 302.

The work of a great many different authors has shown that when anemia is produced by hemolytic poisons the resistance of the blood cells is increased, and it is generally accepted that in the pernicious and hemolytic types of human anemia the resistance of the red cells is increased whereas in secondary anemias it is diminished.

From our experiments we conclude that the red cells of individual sheep show marked variations to laking either by immune serum or by hypotonic salt solution and that resistances by laking by these two agencies are always parallel to each other. These differences are not due to acute hemorrhage. Whether they are due to differences in race or to differences in hygienic conditions (prolonged confinement, immunization with typhoid bacilli) we cannot yet state. During a short period of observation (about a month) in the case of two of the sheep, the cells of each animal were practically constant. There is a slight diminution in the tonicity of the blood serum immediately after an acute hemorrhage: this is possibly due to the fact that the body can more rapidly obtain water than it can salt and other serum constituents. The apparent tonicity of the serum has no relation to the tonicity of the red cells of the individual.

101 (918)

The influence of decerebration on the convulsant action of caffein in frogs.

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It is well-known that destruction of the brain causes increase of reflex action in frogs, especially when they are kept in the cold, and last year I reported that the effect of morphin, which causes in frogs tetanus indistinguishable from that of strychnin, was very markedly increased by decerebration, the effective dose in such frogs being about one tenth of that in normal frogs.

I wish to report now on the result of a study of the effect of decerebration upon the convulsant action of caffein. Caffein salts