change is an increase of the globulins and a decrease of the albumins. Such being the case, it seemed of interest to ascertain whether these modifications affected the rate of coagulability of the blood. Accordingly, the blood of a number of horses which have been immunized for months or years for the purpose of obtaining antisera against diphtheria, tetanus, meningitis, etc., was investigated from this point of view. Oxalated plasma was employed in the coagulation tests. The accompanying table gives the results of this investigation, and shows that the coagulation-time remains fairly constant in spite of marked variations in the proteins, that it is a factor which is not readily disturbed.

| Horse No. | Total Protein. | Euglobulin. | Pseudo-globulin. | Albumin. | Coagulation Time. |
|---|---|---|---|---|---|
| 414 | 6.93 | .78 | 2.74 | 3.27 | 14 min. |
| 508 | 6.15 | .30 | 3.58 | 2.15 | 14 " |
| 536 | 7.49 | .54 | 4.08 | 2.66 | 12 " |
| 542 | 6.49 | .60 | 3.61 | 2.15 | 14 " |
| 566 | 7.22 | .53 | 2.98 | 3.59 | 7 " |
| 593 | 6.93 | .53 | 3.07 | 3.21 | 12 " |
| 596 | 7.20 | .76 | 2.90 | 3.37 | 14 " |
| N. 1 | 7.17 | .61 | 3.42 | 2.97 | 12 " |
| N. 2 | 6.70 | .48 | 3.05 | 2.98 | 15 " |

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## Inhibition of sodium oleate hemolysis and toxicity by cholesterin.

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The hemolytic quality of soap solutions and particularly of sodium oleate has been studied by a number of investigators (Sachs, Meyer, Moore). Meyerstein later showed that the oleate hemolysis could be inhibited by lipoids, cholesterin, serum and organ extracts. Sodium stearate and palmitate are less active than the oleate. Moore has shown that the laking qualities of the soaps are in proportion to the unsaturated bonds of the fatty acids and that by iodine saturation can be inhibited.

The toxicity of soaps was studied by Munk who found that the quantity equal to 0.11 to 0.13 gram oleic acid per kilo body

weight killed rabbits. In his experiments he introduced these quantities very slowly. The heart action was depressed, but continued longer than the respiratory function.

We have given rabbits sodium oleate intravenously. A 5 per cent. solution of anhydrous sodium oleate (Merck) in normal saline was used. The soap solution was injected into the ear veins in a single and rapidly given dose. Doses of 0.1 sodium oleate per kilo appeared very irritating, the muscles of the extremities were thrown into spastic contractions and the animal had convulsive seizures. The ill effects lasted for a minute and a half when the animal would show great depression with rapid breathing. The muscles then became quite limp; within an hour the animal was fully recovered. Doses of 0.13 gram per kilo weight were fatal. These animals would sometimes enter a convulsive seizure before the injection was completely made. The blood of these animals after death was not perceptibly laked and it would appear that in the animal body the quality of laking and that of toxicity were separate. When, however, the solution of sodium oleate was mixed with an equal quantity of cholesterin the toxic qualities were inhibited. This cholesterin sodium oleate mixture was prepared by adding 5 per cent. cholesterin to a previously prepared 5 per cent. solution of sodium oleate in normal saline, and heating in a water bath for several hours. A milky and permanent emulsion was thus obtained which was found to contain no free cholesterin but was filled with great numbers of anisotropic bodies. These bodies are probably cholesterin-sodium-oleate compounds and are not the pure cholesterin esters described by others. This emulsion may be inoculated intravenously in doses containing double the quantity of the lethal sodium oleate. Irritating effects were still observed in spastic muscular contractions but there were transient. The emulsion inoculated intraperitoneally produced some inflammatory reaction with slight fibrin exudate. A single intravenous dose led to no permanent injury in the animal, but repeated inoculations every second day, led to much wasting, so that one animal in a period of twelve days lost 900 grams. Such animals when set at rest rapidly recovered. It is possible that the toxicity of the soap solution may be still further masked by increasing the cholesterin

to saturation. In this state, however, the compound is very gelatinous and hard to handle, while the addition of water for further dilution again leads to dissociation products.

Animals treated with the cholesterin sodium oleate mixture show no evidence of excretion of these substances in the urine. The compound readily circulates in the blood and is not filtered out by the capillaries. The lungs are quite free from change. We have noted, however, that frozen sections of formalin-fixed tissue showed anisotropic globules and an unusual amount of (?) cholesterin spicules in the liver parenchyma. The liver appeared quite yellow and fatty but the fat did not exist as the coarse globular fat of fatty infiltration. In these experiments we did not find the enlarged fatty adrenals as were present in another series in which the cholesterin materials were fed to the animals. The fate of the cholesterin and soaps has up to the present time not been determined.

The same cholesterin sodium oleate emulsion was used to demonstrate the inhibitory qualities of cholesterin upon soap hemolysis. It was found that 0.05 c.c. of a 5 per cent. solution of sodium oleate would hemolyze 1 c.c. of a 1 per cent. suspension of human blood cells in less than 18 hours at room temperature. On the other hand, 1.2 c.c. of the cholesterin sodium oleate compound did not hemolyze a similar quantity of blood. It was likewise found that the addition of normal serum to any mixture of sodium oleate and cholesterin still further inhibited the hemolysis and toxicity of the soap.

It is quite easy to prepare solutions of soap containing different quantities of cholesterin and by this means, observe the inhibitory influences of this substance. We have found that the 5 per cent. solution of sodium oleate in saline with the addition of 5 per cent. pure cholesterin is the most useful for study and is easily handled. The histological changes in tissues resulting from abnormal quantities of fluid cholesterin compounds will be reported upon later. By this combination of soap and cholesterin we have a means of introducing this otherwise inert substance in a fluid state which is assimilable.