TABLE III. Comparison of Avg Daily Intake and Retention of Dietary Nitrogen and Sulfur and Rate of Healing.*

Group #	Nitrogen		Sulfur		Rate of healing index†
	Avg daily intake, mg	Avg daily balance, mg	Avg daily intake, mg	Avg daily balance, mg	
I	266	78	13.2	11.8	16.6
II	78	10	3.6	2.6	10.5
III	269	47	8.2	6.4	12.6
IV	251	56	23.1	14	18.8

* These values indicate daily avg for 21 days after wounding.
† See Fig. 1.

available is increased; (b) increasing the ratio of sulfur to nitrogen by dietary means (by the use of methionine) increases the rate of healing; (c) healing wound tissue contains larger amounts of sulfur than the same tissue when it has not been wounded(21,22).

Thus it seems probable that the rate of healing may be related to, among other factors, the amount of "protein sulfur" retained in excess over that utilized to form normal tissue protein with the retained nitrogen. Since the ratio of nitrogen to sulfur in most tissues is about 16:1, then the healing should be influenced by the amount of retained "protein sulfur," less 1/16 the quantity of retained nitrogen. This might be called the "excess sulfur" which is available for the greater demands of the healing wound tissue. The "excess sulfur" is 156, 44, 75 and 214 mg for the animals in Groups I to IV, respectively. Thus, there appears to be some correlation between the rate of healing and the "excess sulfur" available for the wound.

Summary. Wounded rats were kept on diets containing varying amounts of different proteins and "protein sulfur". There appears to be no relation between the amount of protein nitrogen fed or retained by the rats and the rate of healing. However, the "protein sulfur" retention appears to be correlated with the rate of healing. It is suggested that the amount of retained sulfur in excess over that utilized for normal tissue protein synthesis is an important factor in determining the rate of healing of experimental wounds.

Received April 24, 1951. P.S.E.B.M., 1951, v77.

21. Layton, L. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 570.

22. Sylven, B., *Acta Chir. Scand.*, 1941, v66, 1.

Variations in Proteolytic Activity of Serum of Animals Including Man.* (18760)

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In a previous publication(1) it was shown that there were significant differences in the activation of plasminogen to plasmin, by streptokinase and protamine, in seven species of animals. It was shown that in man only was there a significant increase of streptokinase activated plasmin over the spontaneously and protamine activated plasmin. In monkeys also there was probable though lesser activation by both streptokinase and protamine. It was also observed that the spontaneous activity in some animals was almost as great as the maximal activation by streptokinase in humans.

With this type of reaction it was necessary

* This study was supported by the Damon Runyon Fund for Cancer Research, Institutional Research Grants 34 and 86.

1. Cliffton, E. E., and Downie, G. R., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 559.

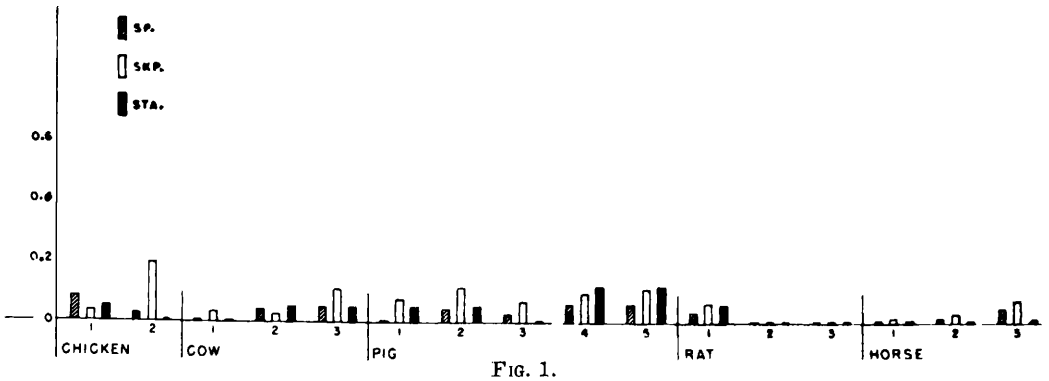

FIG. 1.

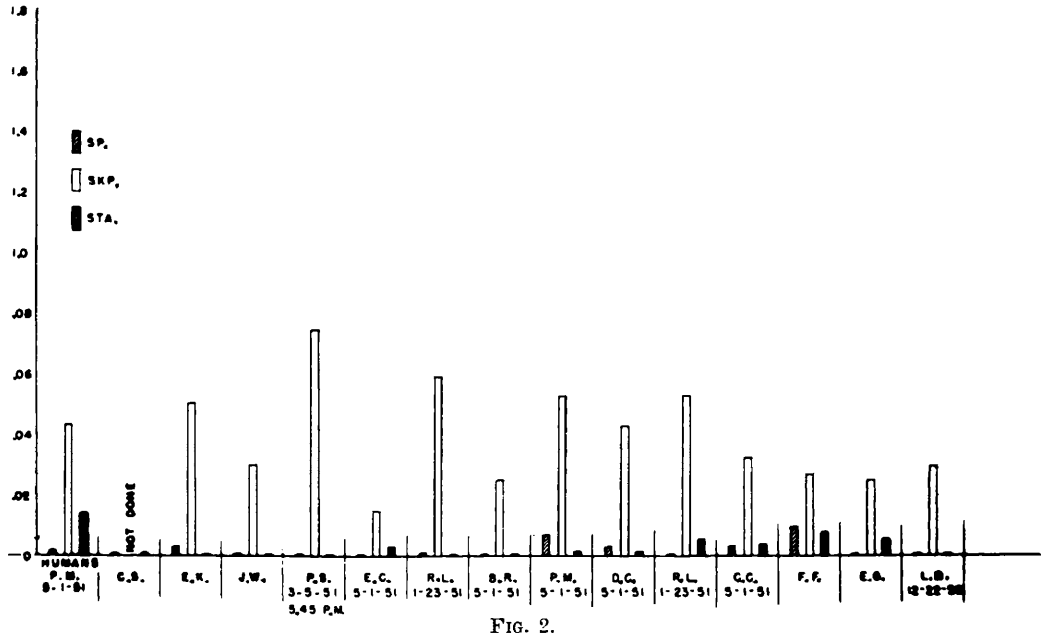

FIG. 2.

to do all activation studies with streptokinase on human serum which limited the experimental approach to the problem. Gerheim *et al.*(2) had previously reported the presence of a staphylokinase which also activated plasminogen(3), and in a later publication(4) they noted a variation in activation of animal sera by staphylokinase different from the activation pattern with streptokinase. They, however, used a fibrinolytic method of determination rather than the proteolytic (casein digestion) method. The present study was undertaken when an active staphylokinase was prepared according to the method of Gerheim, Ferguson, *et al.*(2).

Materials. 1. Plasminogen—prepared by the previously reported modification of Milstone's method(1). 2. Streptokinase (SKP) crude—the filtrate of a 24-hour culture of Group A, Type II, Beta hemolytic streptococci grown in beef heart infusion broth with 0.05% added dextrose. 3. Staphylokinase (STK)—prepared according to the method of

2. Gerheim, E. B., Ferguson, J. H., and Travis, B. L., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 525.

3. Gerheim, E. B., Ferguson, J. H., Travis, B. L., Johnston, C. L., and Boyle, P. W., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 246.

4. Gerheim, E. B., and Ferguson, J. H., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 261.

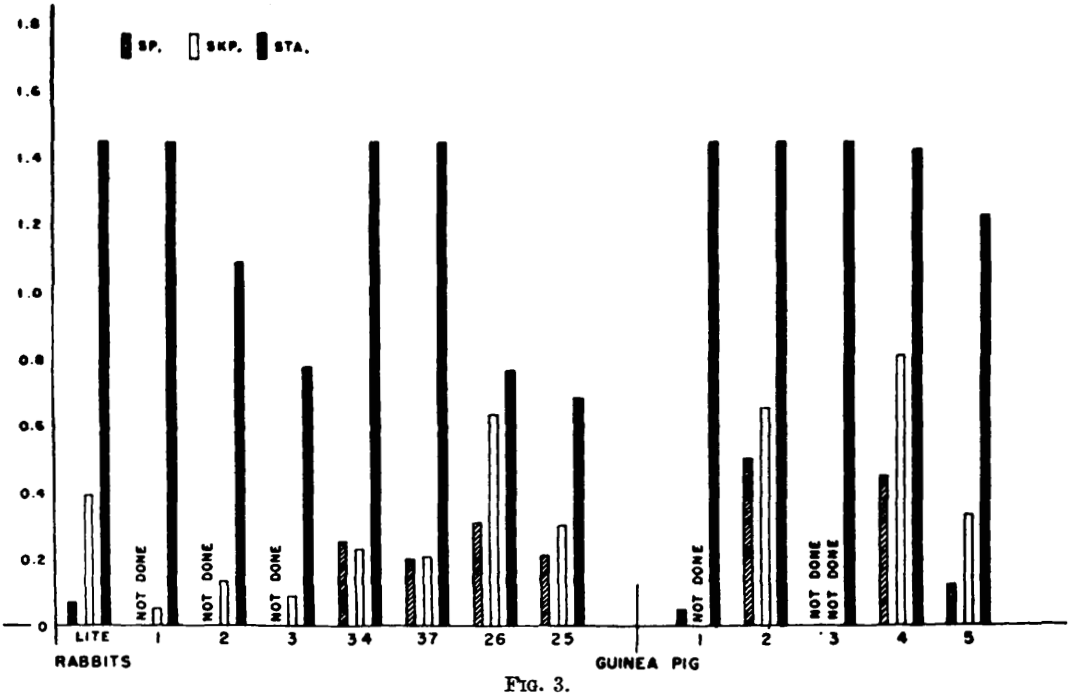

FIG. 3.

Ferguson† from a culture of a virulent *Staphylococcus aureus*(2). The remainder of the materials were the same as those used in the original method(1,5).

Method. As previously reported(5) except that the method of reading the result has been altered as follows: the blue dye, T-1824, is no longer used but a blank containing the reagents is run simultaneously. The color produced by the reagents used is then negated by setting the galvanometer index at 100% transmittance or at O concentration, with the blank cuvette in position. Using this as a reference, the per cent transmission of each aliquot is then read directly. Spontaneous activation and activation by both STK and SKP were tested in the following animals: 15 humans, 4 monkeys (*Macacus rhesus*), 9 dogs, 5 pigs, 3 cows, 3 horses, 8 rabbits, 3 guinea pigs, and 3 rats.

Results. A marked variation in the proteolytic activity of serum produced, as a result of the addition of STK to the euglobulin fraction, was observed in different animals (Fig. 1, 2, 3, and 4).

Horses, cows, pigs, and chickens showed very little activity either spontaneously or by action of SKP or STK (Fig. 1). Human patients once again showed a marked activation by SKP as with our previous series, but very little or no activation by STK except in one instance (P.M., Fig. 2). Even in this case the activation by SKP was 3 times as great as that by STK.

Rabbits, guinea pigs (Fig. 3), monkeys and dogs (Fig. 4) showed the maximum activation by STK with the readings actually off the colorimeter scale in most instances. Four of the 8 rabbits showed the maximum effect and the other 4 showed a marked STK effect. In general the SKP and spontaneous activities of the species were of a similar degree to that previously reported.

† A small quantity of staphylokinase was originally supplied by the kindness of Dr. John H. Ferguson and Dr. Jessica H. Lewis of the Department of Physiology, University of North Carolina. This was used for preliminary studies not reported here. The staphylokinase used in these experiments was prepared in this laboratory with the help of Miss Arline Strouse of the Surgical Bacteriology laboratory.

5. Downie, G. R., and Cliffton, E. E., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 138.

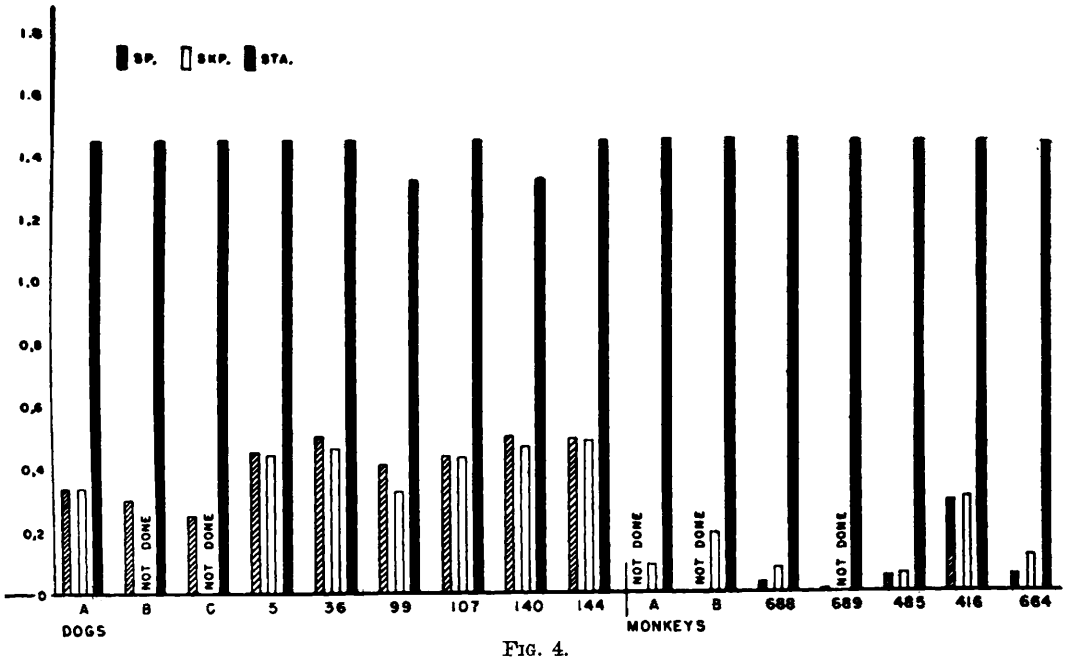

FIG. 4.

Discussion. The species variations in activation of plasminogen by SKP and STK are difficult to explain. One logical concept is that the animals which show no activation have active anti-kinases in their serum either as the result of low grade infections from these organisms or as a part of their normal armamentarium for defense. It is also possible that the plasminogen itself in these instances is resistant to the specific kinase. The present data with an increase of proteolytic activity with STK in dogs, rabbits, and guinea pigs disproves the previous supposition that these animal sera were not activated by SKP because the maximum amount of material available was spontaneously activated(5).

There is one variation from Gerheim and Ferguson's data(4); the lack of significant activation of human plasminogen by STK. However, this may well be due to the fact that they were using a fibrinolysin test and we were using a test of proteolytic activity (casein digestion). Preliminary studies indicate that there is a variation in fibrinolytic and proteolytic activity as produced by STK (data to be published).

The fact that STK activates the plasminogen from sera of the ordinary laboratory animals—monkeys, dogs, guinea pigs and rabbits —makes this an excellent tool for the further study of this enzyme system. Such study was previously markedly limited because SKP (the other rapid and fairly complete activator) activated only human serum plasminogen and not that of ordinary laboratory animals.

Summary. Plasminogen (euglobin fraction of serum) of certain animal species tested—dog, rabbit, guinea pig, and monkey—is activated by a staphylokinase obtained from a culture of virulent *Staphylococcus aureus*. Humans show very little activation with staphylokinase, but definite activation with streptokinase. The species activation pattern with staphylokinase and streptokinase varies markedly. Plasminogen from other animals (horse, cow, pig, and chicken) is activated very slightly, and some not at all, by any activator thus far employed.

Received May 25, 1951. P.S.E.B.M., 1951, v77.