(torulosis). With that material, one could try a combination of tests for serological precipitation upon the supernatant and of tests for Quelling reactions upon the sediment obtained by centrifugation of the spinal fluid, in a manner analogous to the procedures employed with spinal fluids from cases of pneumococcal, meningococcal, and *Hemophilus influenzae* infection.

It should be noted that antiserums of reasonably high potency† are essential for the optimum results both in precipitation of *Cryptococcus* soluble antigens and in Quellung of *Cryptococcus* capsules. Some cross-reactions occur between *Cryptococcus neoformans* and *Torulopsis rotundata,* various other encapsulated saprophytic yeasts, and type 2 pneumococci. These serological inter-relationships, which will be reported in a later paper, would have to be taken into account in the interpretation of precipitation reactions, but could readily be controlled.

Summary. The experiments showed that soluble antigens of the fungus agents occur in the tissues of mice infected with *Cryptococcus neoformans* and with *Sporotrichum schenckii,* and that these antigens are readily detectable by tests with appropriate antiserums. Soluble antigen was found also in the blood of *Cryptococcus*-infected mice.

† It has been our experience that adequate *Cryptococcus* antiserums can be produced with greater regularity and promptness than is indicated in the literature, provided closely spaced injections of relatively large doses of weakly encapsulated strains are employed for immunization(4). The antiserums could readily be produced in the usual hospital laboratory. However, for trials of practical applications of the antiserums, we could supply small samples to clinical workers who have access to materials from cases of human cryptococcosis.

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Variations in Proteolytic Activity of Serum of Animals Including Man.* (17743)

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Under normal conditions the blood plasma or serum of man shows little spontaneous proteolytic or fibrinolytic activity, although Dastré(1) early indicated that such enzymes were present. However, as shown by Delezenne and Pozerski(2) and others(3,4), treatment with chloroform produces definite proteolytic activity. Whether this be due to removal of inhibitor or true activation of the precursor has been a disputed subject. Yamakawa produced activity by use of other substances, including alcohols(5). The most consistent, definite and rapid activator has been streptokinase (6-8), but Gerheim *et al.* have also found an active staphylokinase(9). Grob(10) has

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reviewed the numerous materials which have been reported to result in activity. Milstone(6) was able to separate the proteolytic enzyme precursor which he called plasminogen and Tagnon *et al.*(4) separated the chloroform activated enzyme. Both workers indicated that it was present in the euglobulin fraction of serum. That the plasmin (activated plasminogen) is similar to trypsin in that it is a proteolytic enzyme which affects fibrin, fibrinogen, casein, gelatin, etc. has been proven(4,7). However, it has been shown that they are not identical because of a difference in the optimum pH for activity, quantitative difference in the breakdown of the above substances and tests on specific substrates(7,8). Most studies of the proteolytic action of plasmin have been made by testing its fibrinolytic activity. Increased fibrinolytic activity of the blood has been observed in shock(11,12) after the injection of adrenalin(13), at postmortem after death from severe trauma(14), with peptone shock in dogs(15), after electrically induced convulsions(16), and in liver disease(17,18).

A method for assay of the proteolytic precursor (plasminogen) was developed using a method for separation of plasminogen first suggested by Milstone(6), and a colorimetric estimation of tyrosine produced by digestion of casein. This method was described in detail, with data suggesting activation by protamine(19). Sera of 127 patients were examined, several on more than one occasion. Sera of 22 animals including 6 (*Macacus rhesus*) monkeys, 3 swine, 3 guinea pigs, 2 rabbits, 8 dogs and 1 goat were examined, and variations of the activation pattern between other animals and man were observed.

Results. In the previous report it was noted that with duplicate blood samples satisfactory results with a minimal experimental error were obtained (Table I). Using blood obtained from the same patient on separate days without significant change in their condition very similar results were obtained (Table II).

TABLE I.

Pt.	Sample	S.	P.	O.
M.	1	1.2	.39	.305
	2	1.16	.377	.295
G.	1	1.045	.225	.186
	2	1.08	.21	.197
H.	1	.535	.200	.173
	2	.52	.203	.186

Results obtained with duplicate samples of serum, in mg tyrosine produced. As in all tables: S = Streptokinase activation; P = Protamine activation; O = Spontaneous activation.

TABLE II.

Pt.	Date	Mg tyrosine produced by plasminogen activated by: S.	P.	O.
L.R.	12/ 9/48	.836	.165	.055
	13	.858	.128	.033
J.E.	16	.150	.187	.045
	27	.123	.228	.092
J.N.	2/ 8/49	.274	—	—
	9	.275	.057	.015
M.L.	1/12	.385	.062	.088
	2/ 2	.388	.058	.05
M.B.	24	.706	.087	.035
	3/ 1	.680	.145	.075
B.G.	4	.991	.17	.123
	14	1.020	.188	.105
J.S.	5/ 3	.267	.023	—
	5	.300	.070	.006
	9	.320	.081	.024

Results in mg tyrosine produced obtained with separate samples of blood, drawn from one to 20 days apart. No significant changes in patient's general condition, but no attempt made to draw blood under identical conditions. S, P and O as in Table I.

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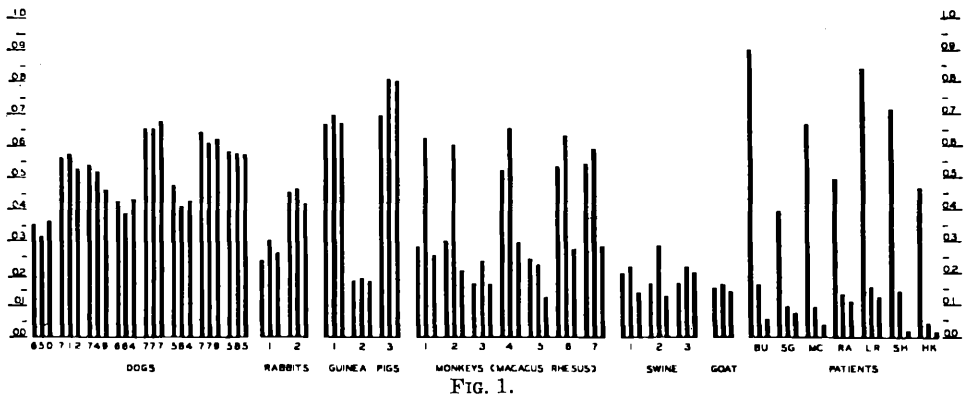

FIG. 1.

Columnar graph to show variations in amount of tyrosine produced by plasmin from animals of several species. A few representative normal men and women have been chosen and all other animals tested have been recorded. Most had repeated tests with similar results. For each individual animal the three columns are from left to right: (1) Streptokinase activator; (2) Protamine activator; (3) No activator (spontaneous). Other tests have been carried out on dogs and monkeys with similar results.

Sera from humans without disease and indeed with all disease except streptococcal infections¶ showed a characteristic activation pattern with streptokinase giving the most rapid and complete activation, protamine giving a definite activation and spontaneous activity being less than either in most instances (Fig. 1). Among the other species the activation pattern differed significantly, with the plasminogen from dogs, guinea pigs, rabbits, swine and one goat, giving almost and at times as much spontaneous activity as with the streptokinase or protamine activated material (Fig. 1). The plasminogen of the monkeys (*Macacus rhesus*) with one exception showed activation by streptokinase similar to that of the human, though to a lesser degree. Activation by protamine, however, was greater in the case of monkeys than with any other species tested. In six species studied, except man, the activity of the protamine-activated plasmin frequently exceeded that of the streptokinase-activated plasmin.

Since the data recorded on the chart were obtained, sera from 12 dogs, 3 monkeys and approximately 125 patients have been tested as a routine reaction by a technician with results essentially the same as those charted.

No explanation for this variation in activation pattern of the proteolytic precursor found in the euglobulin fraction of serum in various animal species is at hand. It is possible that the struggling and anxiety instituted in the animals might possibly cause spontaneous activation, as has been shown to occur to a lesser degree with shock(11,12) and with injection of adrenalin into humans(13). It is conceivable that these animals have all had streptococcal infections and so have developed antibodies to streptokinase. However, their spontaneous activity is of the order of streptokinase activated plasmin in human sera (Fig. 1), which would make it appear we were testing their total activity. The most likely possibility is of course that the euglobulin material itself is significantly different in the different species. Previous study using the fibrinolytic method would tend to confirm this opinion, since variations in the fibrinolytic mechanism of streptokinase from organisms affecting different species have been observed(20,21,22,23,24). This variation is of some importance in the study of the proteolytic-antiproteolytic balance of the serum since much of the experimental work has been done on dogs, guinea pigs and rabbits, in which the mechanism of proteolytic activation

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is apparently different, and so correlation between these results and human reactions must be carefully analyzed. These data would suggest that monkeys should be used for animal experimentation on this complex system.

Summary. An euglobulin fraction of serum or plasma from animals and man contains a proteolytic precursor or active enzyme. The activation pattern of this enzyme precursor in man and monkeys is different from that of dogs, guinea pigs and rabbits.

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Absence of Hemorrhage Inducing Activity in Phthiocol Derivatives with a Basic Nitrogen in Side Chain. (17744)

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In previous reports(1,2) it was shown that 3-hydroxy-1,4-naphthoquinone derivatives, substituted in the 2 position with a hydrocarbon chain of 6 or more carbon atoms, induced a hemorrhagic syndrome in the rat. This syndrome, characterized by a marked hypoprothrombinemia, could be prevented by administration of vitamin K (2-methyl-1,4-naphthoquinone) or K_1 (2-methyl-3-phytyl-1, 4-naphthoquinone), the latter being more active than the synthetic compound. From these findings it was postulated that these phthiocol analogues competed with vitamin K in the processes concerned with prothrombin production.

More recently we have examined 3 phthiocol derivatives* with a basic nitrogen atom in the side chain attached in the 2 position of the naphthoquinone. These derivatives were A 223, 2 - (piperidinomethyl) - 3 - hydroxy-1,4-naphthoquinone, A 1506, 2-(n-butylaminomethyl)-3-hydroxy-1,4-naphthoquinone, and A 1507, 2-(n-decylaminomethyl)-3-hydroxy-1,4-naphthoquinone.

The technics employed in the present study were the same as those used previously(1,2). Male rats, 4-5 weeks old, Sprague-Dawley strain, were used and were fed Purina Dog Chow checkers and tap water *ad libitum*. The drugs, suspended in olive oil, were administered once daily via stomach tube in doses of 400, 800, and 1600 mg/kg. Six rats were given each drug level and the treatments were given for 10 days if the rats survived that long. Prothrombin time determinations(3) were performed on whole blood obtained by cardiac puncture either at the end of the treatment period or when a rat became moribund. At the end of the treatment period all rats were sacrificed and necropsies were performed.

Compound A 223 was found to be about 4 times as toxic as A 1506 and considerably more than 4 times as toxic as A 1507. Two of the 6 rats receiving 400 mg/kg A 223 succumbed; larger doses were uniformly fatal. A 1506 retarded growth at a dose of 800 mg/kg and was lethal to 4 of 6 rats at 1600 mg/kg. No toxicity other than a slight retardation of growth was noted with A 1507 even when doses of 3200 mg/kg were administered. The rats intoxicated with A 223 or A 1506 were usually pale and cyanotic. There was no evidence, however, of the hemorrhagic syndrome encountered in the previous studies. Prothrombin times of both the treated rats and the control animals were all within or close to the normal range (15-18 seconds). Occasionally in the rats receiving A 223 or A 1506 splenic enlargement and a brownish pigmentation of the lungs, heart, and skin were observed at necropsy. It was thought that this pigmenta-

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