TABLE II.
Results of Intracerebral Neutralization Test in Mice Using Colorado Tick Fever Mouse Brain Virus and Russian Spring-Summer Encephalitis, Louping-Ill and Colorado Tick Fever Immune Sera.

Serum	Animal source	LD_{50} titer of Colorado tick fever	LD_{50} doses of virus neutralized by sera
Russian Spring-Summer	guinea pig	$10^{-4.85}$	14
Louping-Ill	" "	$10^{-6.36}$	0
Colorado tick fever	hamster	$10^{-2.40}$	3981
Normal control	guinea pig	$10^{-6.0}$	

very slight or insignificant protective power.

Adaptation of the virus to developing chick embryo. Twenty 7-day-old chick embryos were inoculated by the yolk sac route with 0.25 ml of 1% mouse brain suspension representing the 12th mouse brain passage of the virus. Four living embryos were harvested on the fifth day after inoculation and a 10% suspension of the embryos was inoculated into another lot of 20 7-day-old embryos. Titration of the inoculum by the intracerebral injection of mice resulted in $10^{-3.5}$ LD_{50} titer. From the second passage on, a 10% suspension of living embryos, harvested on the fourth day after inoculation, has been routinely employed as passage inoculum. Up to now the virus has been carried through 12 passages. No deaths have been observed among the inoculated embryos. All attempts to demonstrate cultivable bacteria have been consistently negative. Chick embryo suspension, representing the tenth egg passage of the virus, was used as a source for neutralization tests against Colorado tick fever immune human serum of Dr. Florio (see above). The results of the tests, also summarized in Table I, indicate that the pathogen propagated in the developing chick embryo, seems to be closely related to the agent which caused infection of the human individual.

15466

A Toxic Principle from Progestational Endometrium and Placenta.

CHARLES L. SCHNEIDER. (Introduced by A. J. Cox, Jr.)
From the Department of Pathology, Stanford University School of Medicine, San Francisco, Calif.

Saline extracts of pregnant and pseudopregnant rabbit uteri have been shown to be highly toxic to rabbits when injected intravenously, while similar extracts of muscle, liver and nonpregnant uteri had no comparable effect.[1,2] Saline extracts of placenta have been similarly toxic.[3] The possibility that this induced intoxication might have some relation to toxemia of pregnancy has been expressed.[3-5] A further study of similar tissue extracts was undertaken in order to learn more about the nature of the toxic action and to determine more specifically the origin of the toxic substance. Extracts of different portions of the uterus in different functional states from several animal species, as well as extracts of liver, intestine and skeletal muscle, have been studied by a method for quantitative estimation of the toxic effect.

Method. The tissue was ground in a mortar with sand. The resultant paste was diluted

1 Krichesky, B., and Pollock, W., *Am. J. Physiol.*, 1940, **130**, 319.

2 Krichesky, B., and Mahler, J., *Endocrinology*, 1942, **30**, 616.

3 Obata, I., *J. Immunology*, 1919, **4**, 111.

4 Young, J., *J. Obs. and Gyn. Brit. Emp.*, 1914, **26**, 1.

5 Bartholomew, R. A., and Kracke, R. R., *Am. J. Obs. and Gyn.*, 1932, **24**, 797.

with 0.9% NaCl solution and the sediment was removed by centrifugation. This sediment was again extracted with 0.9% NaCl solution and the separated supernatant fluid was added to the primary extract, which was then neutralized with N/10 NaOH until faintly alkaline. This solution, which was cell free, was adjusted so that 4 cc represented one g of source material; it was then stored at 0°C.

Young white mice were used as test animals. These were obtained from the colony maintained in the laboratory of the Department of Obstetrics and Gynecology at Stanford University. White mice from several other sources (including CFW), brown mice (agouti C_3H) and wild mice gave similar results. No sex difference has been observed.

Trial doses of the extract to be tested were given intravenously into the lateral tail veins of weighed mice until the minimum lethal dose for a 20 g mouse could be estimated by interpolation. The toxicity of the tissue extracts was expressed as the number of minimal lethal doses, or "units," per cubic centimeter. Ordinarily, about 8 mice were used for each assay. Repetition of assays gave results of similar magnitude, which usually checked within 10%. Estimations of toxicity of different dilutions of the same preparation (2.5-40 u/cc) demonstrated an inverse proportion between the dilution of the extract and the toxic effect.

Reaction of the test animals. After intravenous injection of toxic doses of active extracts the mice almost immediately became comatose and apnoeic. Some had generalized convulsions. Most either died promptly or apparently recovered within a few minutes. When an injection was fatal the heart continued to beat after respiratory movements had ceased. In a few animals, clotted blood was found in the mesenteric veins and in the vena cava soon after death. However, these could not be identified as ante-mortem clots, and no other anatomical findings explained the deaths. No hemorrhages or other lesions were seen in the brain. A few animals remained in collapse for as long as a few hours, after which there was death or gradual recovery. An occasional mouse, several hours after apparent recovery, developed violent convulsions and died.

In mice and rats, and apparently also in rabbits and dogs, an increase in tolerance could be built up by repeated injections of toxic extracts. After such preparation, animals survived the injection of 2 to 4 times the fatal dose. Within 2 days the acquired tolerance was completely lost in mice.

Light ether anesthesia during the injection did not modify the course of the reaction. Intramuscular, intraperitoneal and subcutaneous injections of 60 times the fatal intravenous dose failed to produce any response except for temporary reduction in the activity of the animals. An occasional mouse developed convulsions and died after repeated intramuscular injections.

Sources and properties of the toxic substance. Table I summarizes the results of assays of a variety of tissue types. All of the highly toxic extracts were derived from preparations which contained endometrium or placenta. Whether the relatively mild toxic effect of extracts of skeletal muscle on intravenous injection into mice represented the action of a substance identical to that found in the endometrium and placenta has not been determined.

The toxic substance was not extractable in ether, acetone or alcohol. It was lost upon heating extracts to 75°C for 10 minutes, addition of ethyl alcohol to a concentration of 25% or more, or upon acidifying the solution to pH 5. It did not dialyze through a cellophane membrane. Storage of the extracts even at low temperature resulted in a reduction of toxicity, which decreased as much as 50% in 48 hours.

Loss of toxicity of tissue extracts was considerably accelerated at room temperature by the addition of serum of mice or rats; a serum concentration of 5% caused a 50% decrease in toxicity within one hour. Serum of male as well as of female animals was effective. Toxic extracts of skeletal muscle, placenta and endometrium were all inactivated by serum. Heating the serum to 75°C for 10 minutes destroyed its inactivating property.

Several repeated injections of sublethal quantities of toxic extracts did not kill mice

TABLE I.

Approximate Toxicities of Extracts from Different Sources as Determined by the Mouse Assay. Those Designated as "0" contained less than 10 units per gram.

Source of extract	No. of extracts	Units per g of tissue
Endometrium		
Human, secretory phase	4	100
" 3 mo. pregnant	2	200
" menstrual discharge*	6	0
Cow, corpus luteum phase		
portion with papillae	1	50
" without "	1	50
Cow, normal diestrous	2	0
Sheep, " "	4	0
Rat, deciduoma†		
7 days	4	100
12 "	3	0
Endometrium with myometrium		
Rabbit, at estrus (Friedman test animal)	6	100-200
Rabbit, normal virgin uterus	2	0
Rat, pregnant	3	100-200
Deciduoma† uterus		
7 days	7	100-200
12 "	3	100-200
Deciduoma† uterus, with deciduoma core discarded		
7 days	4	100-200
12 "	3	100-200
Normal diestrous	1	0
Mouse, pregnant	3	100-200
" normal diestrous	4	20-100
Placenta, human, at term		
with endometrial layer	5	100-200
fetal portion only	5	100-200
Cow, near term‡	2	100-200
Sheep, " " ‡	4	100-200
Myometrium		
Human, secretory phase	3	0
" 3 mo. pregnant	2	0
Small intestine, rabbit	1	0
Rat	3	0
Mouse	1	0
Liver, rabbit	1	0
Rat	4	0
Mouse	2	0
Skeletal muscle, rabbit	1	0
Rat	3	20
Mouse	1	20

* Extracts of washed menstrual residue were no more active than extracts of the whole discharge.

† Necrosis began 7-8 days following induction and increased progressively.

‡ For anatomical reasons, endometrium remained mixed with cow and sheep placenta.

or rats, and it was found that the animals could tolerate as much as 4 times the lethal dose if the duration of the injection was prolonged to 90 seconds. This suggests that an animal is able to neutralize the toxic property of an injected extract very quickly, perhaps by means of an inactivating effect of plasma. Because of this rapid inactivation of the toxic property, it is necessary in assaying the material to make injections quickly and to avoid backflow of blood into the injecting syringe.

Discussion. Although the nature of the toxic principle found in extracts of endometrium or of placenta has not been determined, the properties are consistent with those of a protein. The toxic factor may be regarded as a specific substance or group of substances since it was not obtained by extraction of a number of other tissues and since it is rapidly inactivated by serum. It is probable that the substance described here is the same as that described as histamine-like by Krichesky and Pollock.[1] The properties of the material, particularly its failure to dialyze, support their conclusion that the substance is not histamine itself.

Since extracts prepared from myometrium were not toxic and since endometrium or placenta was present in all of the sources which produced strongly toxic extracts, it is probable that endometrium and placenta were the principal source of the toxic substance. Endometrium under progesterone influence, such as that in the secretory phase of the menstrual cycle, pseudo-pregnancy, or pregnancy, regularly produced a more toxic extract than endometrium in other physiological states.

It is doubtful whether the substance described here is the same as the menstrual toxin of Schick[6] or of Macht and Lubin.[7] Subcutaneous injection of extracts of menstrual discharge are reported by Smith and Smith[8] to result in local inflammation and in delayed death of injected animals. Extracts of menstrual discharge prepared and administered by the methods described here did not cause death in mice. However, it is possible that a toxic substance from the endometrium may have been inactivated in the discharge by the admixed blood.

6 Schick, B., *Wien. Klin. Wchschr.*, 1920, **33**, 395.

7 Macht, D. I., and Lubin, D. S., *J. Pharmacol. and Exp. Therap.*, 1924, **22**, 413.

8 Smith, O. W., and Smith, G. S., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 285.

Summary and Conclusions. Toxic material, which causes death of mice on intravenous injection, can be extracted from progestational endometrium and from placenta of several mammalian species. Small amounts of material with a similar effect are present in extracts of skeletal muscle. Quantitative estimation of the toxicity of a variety of tissue extracts has been made. Addition of blood or serum rapidly diminishes this toxic property.

Special appreciation is due Miss Carol Spaulding for assistance in conducting this investigation.

15467

Liver Necrosis in Mice Following Injection of Toxic Extracts of Progestational Endometrium and of Placenta.

CHARLES L. SCHNEIDER. (Introduced by A. J. Cox, Jr.)

From the Department of Pathology, Stanford University School of Medicine, San Francisco, Calif.

During a study of the action of toxic extracts of endometrium and of placenta[1] a number of treated mice were found to have focal necrosis of the liver. Liver lesions have appeared in 174 of the animals; this represents an incidence of about 30% in mice which received sublethal amounts of toxic extract injected intravenously. The animals ranged from 1½ to 2 months of age. The lesions have been studied further by examining the livers of treated animals at different intervals following the injections.

Results. Within the first hour after injection, irregular areas of discoloration could be seen through the capsule of the liver. At first they were dark red, but after 24 hours they were usually paler than the adjacent liver tissue, and became sharply defined. Large lesions often presented a mottled appearance, with darker spots on a pale background (Fig. 1). The lesions were usually near the periphery of the lobes, and their size and number were variable. Usually the altered zones were small, and they occurred most frequently in the large left lobe, but all lobes were involved when the lesions were extensive. Occasionally, with massive lesions, there were fibrinous adhesions of the involved parts to adjacent structures. Small lesions were invariably covered by a smooth surface, although they were usually situated just beneath the liver capsule.

Microscopically there was dilatation of the hepatic sinusoids in the earliest lesions recognized, and erythrocytes were sometimes seen outside of vessels. Within 3 hours vacuolation of hepatic cells could be seen (Fig. 2) in the altered regions, and the nuclei were sometimes small and deeply stained. After 17 to 20 hours much of the cell structure had been destroyed (Fig. 3) in sharply demarcated zones, bordering which the adjacent hepatic cells were sometimes unusually deeply stained. At this stage there were fairly numerous polymorphonuclear leucocytes, particularly near the margin of the lesions.

The lesions had no constant position in the liver lobules. When the lesions were minimal, involving only small groups of cells, they were mid-zonal distribution. Sometimes entire lobules were necrotic, and the lesions commonly extended throughout several lobules or parts of several lobules without apparent predilection for any part.

In several animals there were tiny pedunculated lobes of liver tissue protruding caudally near the right kidney. These all showed necrosis in treated animals, even in the absence of lesions elsewhere. Several recent thrombi in vessels within the narrow stalk which attached these structures to the

[1] Schneider, C. L., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 322.