volume at weights below 100 g and somewhat above 50% at high weights. This confirms the impression acquired incidentally in this laboratory, in the process of accumulating data in numerous other rat experiments, that an indication of the direction of change in actual blood volume can be obtained by measuring the "drawn blood" volume.

Summary. 1. Blood and plasma volumes have been determined in the normal albino rat, and have been found to be approximately related to a power of body weight, the deviation being ascribed to deposition of avascular fat as the rat matures. There appeared to be no significant difference in this regard between the sexes.

2. An estimation of blood volume changes may be made from the "drawn blood" volumes.

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Effect of Chloroform on the Antitryptic Activity of Blood Plasma.

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It was shown in a previous paper[1] that treatment of plasma with 10% chloroform, decreased its hypertensinase activity instead of increasing it. This was a remarkable finding because: (a) hypertensin is a very sensitive reagent to test for proteolytic activity, and (b) the characteristic clotting and fibrinolytic properties of chloroform treated plasma have been attributed to an increased tryptic activity.

Previous experiments have also shown that hypertensin is an excellent substrate for the detection of antiproteolytic substances. Using hypertensin as a reagent, we have been able to show that plasma protects hypertensin against the destructive action of trypsin and to a lesser degree from that of chymotrypsin. On the contrary, the hypertensinase activity of crystalline carboxypeptidase and pure aminopeptidase (from yeast) remained unaffected (Croxatto and Croxatto[2]).

The degree of protection afforded by plasma, against the destruction of hypertensin by an appropriate dose of trypsin, may be used as a measure of its antitryptic activity. By using a technique based on this assumption, we have studied the effect of chloroform treatment on the antitryptic activity of plasma.

Experimental. Dog, ox, horse and human plasmas were treated with 10% chloroform, with the technique already described. Chloroform was separated by centrifuging and vacuum distillation, usually after 24 hours contact with the plasma. Aliquots of treated and untreated plasma were added to hypertensin (3 units) plus 0.1 ml of a solution of crystalline trypsin. A freshly prepared solution of one mg of trypsin* per milliliter of 0.9% sodium chloride was used. The amount of this solution used (0.1 ml) was enough to produce more than 80% destruction of hypertensin in 2 hours, with no plasma present.

The action of plasma on hypertensin, in absence of trypsin, was also tested. All experiments were carried out at pH 7.4, obtained by adding 0.2 ml of a solution of 0.02N sodium phosphate buffer, pH 7.4. The incubation period was of 2 hours at a temperature of 37°C.

The hypertensin activity present after incubation was determined by injecting the solutions into the femoral vein of a cat, under Dial anesthesia and recording with the

[1] Sainz, N., and Croxatto, H., *Bol. Soc. Biol.*, Santiago, Chile, **1944, 2, 261.**

[2] Croxatto, H., and Croxatto, R., *Bol. Soc. Biol.*, Santiago, Chile, in press.

* Plant's crystalline trypsin containing 85% of magnesium sulfate.

usual technique the changes in blood pressure in the carotid artery. All solutions were boiled and then centrifuged, using supernatant. This treatment eliminates completely the secondary effects of foreign proteins and trypsin on the blood pressure. The sensitivity of the animal was checked, from time to time, with a standard solution of hypertensin.

The amount of plasma, not treated with chloroform, necessary to afford a 50% protection (1.5 units of hypertensin) varied between 0.04 and 0.06 ml. The hypertensinase activity of such a volume of plasma was negligible.

Results. As shown in Fig. 1, the treatment of plasma with chloroform decreases greatly its antitryptic activity. Although chloroform treatment does not eliminate completely the antitryptic activity, the decrease is larger after 24 than after one hour contact. The antitryptic activity of 0.5 ml of 24 hours treated plasma is approximately equivalent to that of 0.05 ml of untreated plasma.

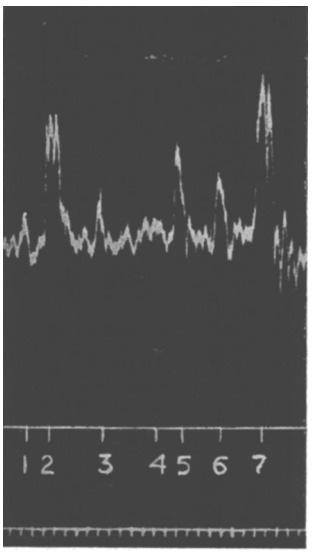

FIG. 1.

Discussion. Our results favor the hypothesis of an inactivation, by the chloroform treatment, of one or several substances acting as inhibitors of the pancreatic trypsin. Heparin cannot be considered as such an inhibitor because of not appreciably influencing the inactivation of hypertensin and pepsitensin by trypsin, whether plasma is present or not (Marsano, Croxatto, Croxatto[3]).

The clotting and proteolytic activities of chloroform treated plasma may be due to an inhibition of antitryptic substances. In fact, the addition of crystalline antitrypsin extracted from soy bean meal, counteracts the clotting and fibrinolytic action of chloroform treated plasma and certain snake venoms (H. Croxatto[4]).

The changes produced by the chloroform treatment are very complex and difficult to interpret. Several enzymatic activities of plasma are disturbed (Sainz and Croxatto[1]). and the interaction of hypertensinogen, renin, and hypertensinase is changed (Croxatto and Croxatto[2]). Furthermore, no explanation can be given of why hypertensin is destroyed more easily by non-treated plasma than by plasma treated with chloroform, while the proteolytic activity is increased by this treatment, as shown by the solubilization of the blood clot.

Summary. Treatment of human, dog, ox, and horse plasmas with 10% chloroform, decreases their antitryptic properties.† These properties were measured using hypertensin as substrate.

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3 Marsano, A., Croxatto, R., and Croxatto, H., *Bol. Soc. Biol.*, Santiago, Chile, 1945, **2**, 350.

4 Croxatto, H., *Rev. Soc. argent. Biol.*, 1946, **22**, 477.

† While this work was in course of publication, a paper by L. R. Christensen appeared in *J. Gen. Physiol.*, 1946, **30**, 149, where he states that chloroform treatment of serum produces an immediate inactivation of "protease inhibitor."