examination showed the presence of methæmoglobin. Estimations of methæmoglobin and total hæmoglobin by the method of Evelyn and Malloy[8] have been carried out on a number of cases and typical results from three affected sheep are set out in Table 1, with normal levels for comparison.

Table 1. Total Hæmoglobins, Methæmoglobin and Copper-Levels in Whole Blood in Chronic Copper Poisoning

	Blood copper (μgm./100 ml.)	Total hæmoglobins (gm./100 ml.)	Methæmoglobin (gm./100 ml.)
Case 1	1,210	11·5	3·5
Case 2	1,480	8·8	3·6
Case 3	1,000	15·5	3·8
Normal sheep	170	10 to 12	0·1

Copper-levels in blood were extremely high and methæmoglobin constituted 25 per cent to 35 per cent of the total hæmoglobin present. The methæmoglobinæmia was mainly intracorpuscular, but since hæmolysis was taking place, hæmoglobin and methæmoglobin were being released into the plasma, the proportions being approximately the same as occurring in whole blood, for example, plasma total hæmoglobins 2·2 gm. per 100 ml.; methæmoglobin 0·8 gm. per 100 ml. (36 per cent).

The pathological significance of these findings is being investigated.

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Release of Heparin from the Mast Cells of the Rat

Mast cells are an important source of tissue heparin and of histamine[1,2]. Histamine is known to be released following disruption of mast cells[3] and in the dog there is an associated release of heparin[4]. In other animals it has not been possible to detect liberation of heparin coincident with release of histamine.

The liberation of heparin from rat mast cells has been investigated *in vitro* following disruption of the mast cells. Cells were obtained from the peritoneal cavity of the rat by a method already described[3]. Mast cells were separated from other peritoneal cavity cells by centrifugation for 30 min. at 0° C. over a column of bovine albumin of 1·109 specific gravity. The mast cells passed through the albumin column and were collected at the bottom of the centrifuge tube. They were washed in physiological saline solution and suspended in saline solution in a concentration of approximately 0·5 million mast cells per ml. of solution. The mast cells in 1 ml. of suspension were disrupted by the following methods: (1) alternately freezing and thawing three times; (2) mechanical disruption in a piston and cylinder type homogenizer; (3) addition of compound 48/80 to give a final concentration of 2×10^{-6} mgm./ml.; (4) osmotic disruption with distilled water; (5) disruption by contact with an antigen–antibody mixture as already described[3]. This mixture consisted of 1 vol. of a 25 per cent suspension of human red cells in physiological saline solution and 5 vol. fresh rat antiserum at 37° C. and *p*H 7. After disruption of the mast cells, the preparations were centrifuged and the supernatant solutions assayed for antithrombin activity by the method of Monkhouse and Jaques[5].

The only supernatant solution of the disrupted mast cells found to have any antithrombin activity was the preparation that had been frozen and thawed three times. Activity equivalent to approximately 3·5 I.U. of heparin was released into the supernatant solution for each million mast cells frozen and thawed. Antithrombin activity remained uniform throughout the supernatant after centrifugation for 1 hr. at 28,000*g* in a 'Spinco' ultra-centrifuge. A residual small amount of activity, less than the equivalent of 0·5 I.U. heparin per million mast cells, was found in the deposit of the disrupted cells.

If the deposits of the other four solutions were digested with trypsin at *p*H 8·0 and then neutralized, the solutions showed antithrombin activity equivalent to approximately 4 I.U. heparin per million mast cells. This value agreed with the antithrombin content of untreated mast cells. As with heparin, the activity was readily neutralized by small amounts of protamine sulphate.

Microscope examination of stained smears of the disrupted mast cells revealed disruption of the granules only in the preparations which had been frozen and thawed.

It may be concluded that the antithrombin present in rat mast cells is not released into the surrounding solution following disruption of these cells *in vitro* unless the granules also are disrupted. This is in contrast to the results with histamine, which is known to be released from disrupted rat mast cells even though the granules remain intact.

Compound 40/80 used in these experiments was kindly made available by Burroughs Wellcome and Company (Aust.), Ltd.

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Mast Cells in Photosensitivity Reactions

In recent work[1] it was found that cells obtained by washing out the rat's peritoneal cavity with saline could be rendered photosensitive. Exposure to light for a period too brief to affect leucocytes and serosal cells caused the granules of sensitized mast cells to lose their basophilic staining properties, after which they became refractory to the action of the histamine-releaser, compound 48/80. Mast cells reacted in this way even after being separated from the other cells by differential centrifugation.

In order to determine whether histamine had actually been liberated when mast cells lost their