

NOTE ON "LEECH-EXTRACT" AND ITS ACTION ON BLOOD. BY W. L. DICKINSON, M.B., M.R.C.P., *Gonville and Caius College.*

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It was established by Haycraft¹ that a watery extract of the anterior part of the medicinal leech has, when mixed with shed blood or injected into the circulation, a strong delaying or preventive action upon clotting. He found further that this extract may be prepared of unimpaired strength from leeches which have been exposed to the action of alcohol long enough to coagulate the ordinary proteids, that it is uninjured by boiling, and that it apparently destroys the fibrin-ferment. My experiments have been made with a view of isolating the active principle of this extract and of studying its action.

In the way of its isolation the peculiar difficulty is interposed that the glandular structures which are almost certainly the source of the secretion in question occur in a scattered unicellular form, and are not separable from the other tissues by dissection². And from the tissues of the leech in all regions of the body is derived a proteid of the albumose group from which I have failed to distinguish or separate the special substance which prevents the clotting of blood.

The method of obtaining the extract is to cut off the anterior part of the animal as far back as the commencement of the crop, place under alcohol for not less than several days³, and finally treat with water or normal salt solution. Thus obtained, the extract is tinged with pigment, but if the strength is such that one leech head corresponds to 5 or 10 c.c. of fluid, it is of convenient working power while there is not enough pigment present to obscure the chemical reactions.

¹ *Proc. Roy. Soc.* Vol. xxxvi.

² See Bourne, *Quar. Journ. Mic. Sci.* Vol. xxiv. p. 431, for description of glands in *Hirudo*.

³ No length of exposure to alcohol seems to impair its activity. I have obtained a powerful extract from leeches which had lain under alcohol (95 p.c.) for 3½ years.

General properties of leech-extract.

It is neutral to litmus-paper.

Its specific gravity is not appreciably higher than that of the medium used for extracting.

Boiling causes no precipitate.

Alkalies cause no precipitate.

A trace of acetic acid causes cloudiness readily soluble in excess.

Glacial or strong acetic acid cause no precipitate in salt-free, but a copious cloud in salt-saturated extracts.

Nitric acid in the cold causes a decided precipitate, soluble on boiling and reappearing on cooling.

(This reaction can always be distinctly recognised, but it is only with certain proportions of HNO_3 that the fluid is perfectly clear on boiling.)

Saturation with Sodium Chloride or Magnesium Sulphate causes no precipitate.

Saturation with Ammonium Sulphate causes a small precipitate, after which practically no proteid remains in solution.

Dialysis of all salts away from the extract causes no precipitate and no loss of power.

(The dialysate usually contains enough proteid to give a faint xantho-proteid reaction on concentration, but has no anti-clotting power.)

Copper Sulphate, Lead Acetate, and Mercuric Chloride give precipitates insoluble in excess of these reagents.

A pink colour is produced by Copper Sulphate and caustic soda (biuret), best seen when a considerable depth of fluid is employed.

It will be seen from these reactions that leech-extract contains a proteid with some features in common with Kühne's proto- and others with deutero-albumose. I have not found any chemical difference in extracts of the anterior active and hinder inactive parts of the leech, but the amount of albumose (as judged by the ppt. with HNO_3 in comparative extracts of equal weighed quantities of the anterior and posterior parts respectively) is somewhat greater in the former than in the latter.

The precipitate caused by saturation with $(\text{NH}_4)_2\text{SO}_4$ carries with it the whole of the active principle, and partial saturation, e.g. with 20 p.c. $(\text{NH}_4)_2\text{SO}_4$ precipitates some of the albumose and a proportionate quantity of active principle.

In one experiment saturation was three times carried out; the ppt. being each time collected on a filter, washed in saturated solution of $(\text{NH}_4)_2\text{SO}_4$, and redissolved in distilled water: the solution of the ppt. from the third saturation retained its original power, while the filtrates after apodialysis of salt and concentration, possessed no anti-clotting power whatever.

It is evident therefore that ordinary means do not suffice to distinguish between the active principle and the proteid; and from the fact that the anterior part of the leech is relatively rich in albumose it is probable that the active principle is itself a substance of this nature, rather than merely difficult to separate therefrom.

Characters of plasma prevented from clotting by leech-extract.

Leech-extract is equally efficacious whether injected into the circulation or mixed with the blood as it flows from the vessels. Successive injections have a constant effect—i.e. if, a short time after the blood of an animal injected with the leech-extract has regained the power of natural clotting, a second or third injection be made, the power of clotting is again lost as at first. In both these respects a contrast is presented to the action of "peptone."

Blood of the rabbit, cat, sheep, dog, ox, horse, and of man kept fluid by leech-extract displays the same general characters. The haemoglobin is as a rule undissolved, and the corpuscles subside leaving a clear plasma. If the proportion of extract is insufficient to preserve permanent fluidity a clot commences in the corpuscular sediment and gradually extends. The fibrin thus formed is of normal quality, i.e. not soluble with appreciable rapidity in 5 p.c. solution of NaCl nor in very dilute alkali.

Clotting cannot be induced by CO_2 , nor by neutralisation with dilute acetic acid.

Dilution of the clear plasma to any extent is without effect, but dilution with distilled water of a plasma from which the corpuscles have not been removed will sometimes provoke slow clotting.

Clotting can always be induced by fibrin-ferment in sufficient quantity (5 p.c. NaCl extract of fibrin).

The fibrinogen is unaffected. Heat-coagulation occurs readily at the ordinary temperature of $56^\circ\text{C}.$; and the substance can be precipitated in the usual manner by half-saturation with NaCl. The precipitate thus obtained is very copious, more so than is the case in MgSO_4 -plasma; and after one precipitation the fibrinogen when redissolved

has no tendency to clot spontaneously but can be readily made to clot with a small amount of added ferment—after a second precipitation it clots with a mere trace of ferment.

Horse's blood needs a very small admixture of leech-extract to keep it fluid—about in the proportion of one leech head to 100 c.c. of blood¹.

By freezing or prolonged cooling I have not been able to obtain the precipitate of discoid bodies to which Wooldridge gave the name of Fibrinogen-A: leech-extract—plasma remains under these conditions perfectly clear.

Action of leech-extract upon fibrin-ferment.

Haycraft found that fibrin which had been soaked in leech-extract failed to yield ferment when subsequently treated with 8 p.c. NaCl solution, and concluded that the ferment had been destroyed. I have repeated Haycraft's experiment with nearly the same result.

EXPERIMENT. Fresh washed fibrin was divided into two equal parts by weight, the one being placed for 12 hours in a strong leech-extract made with distilled water, the other in a similar quantity of distilled water. Each was then dried between blotting-paper and thoroughly washed in a large quantity of distilled water many times changed. Upon now treating the two masses of fibrin with equal quantities of 5 p.c. NaCl solution it was found that that which had been soaked in leech-extract yielded a mere trace of ferment power, whereas from the other was obtained a ferment solution of great strength.

10 c.c. dilute plasma were placed in each of two tubes.

A + 10 drops salt-extract of fibrin soaked in leech-extract

clotted feebly in 18 hours.

B + 10 drops salt-extract of fibrin soaked in distilled water

clotted firmly in 3 minutes.

Another method employed of testing the destructive action upon fibrin-ferment was to mix together solutions of known strength. I found that if such proportions of leech-extract and fibrin-ferment, as would when added simultaneously to dilute plasma allow clotting to take place slowly, were previously mixed together for some time, the delay in clotting was always much greater.

EXPERIMENT. 4 c.c. leech-extract were mixed with 40 c.c. solution of fibrin-ferment and placed in a water-bath at 38° C. for an hour.

¹ Thus it seems that when it is desired to isolate fibrinogen or to preserve the blood fluid for other purposes, the employment of leech-extract is more advantageous than the older methods.

10 c.c. dilute plasma were placed in each of two tubes.

A + one drop leech-ext. + 10 drops sol. of fib.-fer. not previously mixed
began to clot in 39 minutes.

B + 11 drops of the mixture

was still fluid after 5 hours.

When a strong solution of fibrin-ferment made by extracting fresh fibrin with a suitable strength of neutral salt has been subjected to the action of a sufficient quantity of leech-extract, it is not possible, as ordinarily, to precipitate the ferment in an active condition by means of saturation with MgSO_4 .

(In a salt-extract of fibrin the ferment is closely associated with the dissolved globulin, and both ferment and globulin are completely precipitable by saturation with MgSO_4 : the active principle of leech-extract, on the other hand, is not precipitable by this salt.)

From a mixture of extract of fibrin and leech-extract the globulin may be precipitated in the usual way, but the precipitate is devoid of ferment-power, thus affording a further proof that the ferment has actually suffered destruction.

EXPERIMENT. 200 c.c. 5 p.c. MgSO_4 extract of fresh washed fibrin were mixed with 20 c.c. leech-extract, and placed for half-an-hour in a water-bath at 38° C.

A control was supplied by a similar mixture in which the leech-extract was replaced by water.

The mixture was then saturated with MgSO_4 , the precipitate collected on a filter, washed in saturated solution of MgSO_4 , and redissolved in distilled water. This process was performed three times—the control being similarly treated. The solutions were ultimately freed from excess of salt by dialysis and their ferment-power was tested.

Horse's MgSO_4 -plasma.

A. diluted 10 times with solution of ppt. from control mixture
clotted in 40 minutes.

B. diluted 10 times with sol. of ppt. from mixture containing leech-extract
failed to clot in 24 hours.

Although this solution had no ferment-power, it contained a considerable quantity of a globulin which resembled that described by Halliburton as cell-globulin β and considered by him to be identical with the fibrin-ferment¹. It coagulated at 75°—80° C., and when

¹ This *Journal*, Vol. ix.

treated with NaCl to the extent of 10 per cent. its coagulation-point was lowered to 60—65° C.

If then it is possible to destroy the fibrino-plastic power of cell-globulin by means of leech-extract and subsequently to separate it in an apparently unchanged condition, a strong argument is afforded for the non-identity of the ferment and the proteid. Upon this consideration it seemed desirable to repeat the last experiment with a solution of cell-globulin prepared according to Halliburton's directions.

EXPERIMENT. A quantity of lymphoid tissue was extracted with $\frac{1}{10}$ -saturated solution of sodium sulphate, and a fairly pure solution of cell-globulin thus obtained. The fluid was slightly viscid, probably from the presence of some nucleo-albumin, and hence NaCl was chosen as the precipitant instead of MgSO_4 .

200 c.c. of this fluid were mixed with 10 c.c. leech-extract and placed in a water-bath at 40° C. for half-an-hour. The mixture was then saturated with NaCl, the ppt. collected, washed, redissolved and freed from excess of salt by dialysis—a control mixture being similarly treated. This solution contained traces of a globulin which opalesced at 50—55° C., and larger quantities of cell-globulin β which coagulated at 80° C. When to a portion of the solution 10 per cent. NaCl was added the coagulation-point of the latter globulin was about 65°C.

Horse's MgSO_4 -plasma.

- A. diluted 10 times with solution of ppt. from control mixture
clotted in 2 hours 15 minutes.
- B. diluted 10 times with sol. of ppt. from mixture containing leech-extract
failed to clot in 48 hours.

It may be objected to the above experiment that a single precipitation of the globulin was not enough to ensure the complete absence of the active principle of leech-extract, and that the failure to cause clotting may have been due to contamination with the latter; but to this it may be answered that the quantity of leech-extract employed was a minimal one, and it is in the highest degree improbable that enough was mechanically carried down with the precipitate to materially influence its action.

Hence there seems to be ground for concluding that cell-globulin may be deprived of fibrino-plastic power without alteration in its physico-chemical qualities.

Literature of Leech-extract.

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