THE PRESSOR PRINCIPLES OF PLACENTAL EXTRACTS. By OTTO ROSENHEIM.

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Some time ago Dixon and Taylor[1] made the observation that alcoholic extracts of human placenta contained substances which on intravenous injection produced a rise of blood-pressure and contraction of the pregnant uterus. I undertook the examination of such extracts with the view of isolating the active principles.

Dixon and Taylor's method of preparing these extracts consisted in mincing fresh human placenta, covering it with absolute alcohol and filtering after some time. The filtrate was evaporated and taken up again with absolute alcohol. The residue of the last extract, dissolved in saline solution, was injected intravenously[2].

As the effect of the injections seemed to suggest the presence of adrenaline or a similar substance, the first question to decide was the identity or otherwise of the pressor principle with adrenaline.

For this purpose the alcoholic extracts of fifty-two placentæ, prepared in the way described above, were used. The physiological activity of the extract was established by experiment[3]. Twelve litres of the dark brown extract, which possessed a characteristic odour, were evaporated *in vacuo*, the reaction being kept acid. The residue was worked up according to the well-known methods for the isolation of adrenaline (Takamine, Aldrich, Abel, etc.). All attempts to isolate adrenaline gave negative results. As this result might possibly have been due to the influence of oxidation, the experiment was repeated with 1 kg. of fresh placenta, the extractions being made with absolute alcohol acidified with trichloracetic acid in an atmosphere of hydrogen. The results were also completely negative. The purified extracts did not show any of the colour reactions of adrenaline.

These experiments therefore established the fact that the active principle was not identical with adrenaline.

[1] *Brit. Med. Journ.* II. p. 1150. 1907.

[2] *Münch. med. Woch.* LIV. p. 1616. 1907. *Internat. Congr. of Physiol.*, Heidelberg, 1907.

[3] The material for this investigation had been collected and kindly put at my disposal by Dr Taylor. The physiological experiments have been performed in conjunction with Dr Dixon.

Conditions necessary for the formation of the pressor principle.

Before proceeding further it was thought advisable to find the most suitable conditions for the extraction of the pressor substance. A large number of experiments were performed for this purpose. The extracts were always evaporated *in vacuo* and their physiological activity tested in the usual way on pithed animals. In every case the minced fresh placentæ were mixed with an equal volume of absolute alcohol to prevent putrefaction and extracted in one of the following ways: (1) with absolute alcohol alone; (2) with alcohol + 1 % acetic acid; (3) with alcohol + 1 % sodium carbonate; (4) residue of (3) with acid alcohol; (5) in a hydrogen atmosphere (*a*) with acid alcohol, (*b*) with alkaline alcohol. The time of extraction was varied under the above conditions from 24 hours to twelve days.

The surprising outcome of these experiments was that all the extracts made under these conditions were found to be devoid of any pressor principle, thus showing clearly that these substances are not present in the normal human placenta.

To find the explanation for this discrepancy from previous results, one has to remember that the essential difference in the preparation of the above inactive extracts and those prepared according to Dixon and Taylor, is the amount of alcohol used, which in the latter case may not have been sufficient to prevent autolytic or putrefactive processes.

The following experiments were carried out to decide this question:

Five fresh human placentæ were minced and divided into two equal parts.

A. 1000 c.c. of the minced material were mixed with 1 litre of absolute alcohol and kept at room temperature for four days. Cultures in broth and gelatin were made at the end of this period and the extract worked up in the usual way. Result: absence of pressor substance. No growth in cultures.

B. 1000 c.c. of the same material were mixed and partially covered by absolute alcohol; 100 c.c. of the latter were necessary. The mixture was kept and worked up exactly as in A. Result: strong pressor effect. Abundant bacterial growth in both culture materials.

These experiments, which were repeated several times with identical results, made it evident that the formation of the pressor substances is connected with the presence of micro-organisms. The following experiment showed directly that a strongly active extract is obtained by allowing fresh placenta to undergo putrefaction freely.

Five placentæ were minced and the whole divided into two parts.

A. 1000 c.c. of the material were kept in the incubator at 36° C. overnight. Result: intense putrefaction. Extract strongly pressor.

B. 1000 c.c. of the same material, mixed with 1 litre of absolute alcohol, were left standing at room temperature. Result: extract inactive.

In view of the abundance of various enzymes in human placenta[1], the possible share of these in the formation of the pressor principles had to be considered. Experiments were therefore carried out in which the usual precautions against bacterial infection were observed. Cultures in broth and gelatin at the end of the experiments showed the absence of micro-organisms. Autolysis was allowed to proceed under the following conditions:

1. 1000 c.c. fresh minced placentæ were shaken up with 1 litre of saline solution, saturated with chloroform. Kept for four days at room temperature.

2. The same experiment was carried out, the mixture being kept at 36° C. for sixteen days.

3. One placenta was transferred directly after birth into 1 litre of saline solution, saturated and covered with toluol. Kept at room temperature for four days.

4. One placenta was transferred directly after birth into absolute alcohol and kept for four days. At the end of this time the alcohol had only partially penetrated into the tissues and autolysis had obviously taken place in the interior of the organ.

Extracts of all the above were prepared in the usual way, bacterial action being guarded against during their preparation. Physiological experiment proved in every instance the total absence of a pressor substance.

These results therefore show clearly that under conditions which freely permit autolytic processes to go on, the active principle is not produced.

As a final proof of the putrefactive origin of the pressor principle; its absence in the fresh placenta; and its non-formation during autolysis a large quantity of fresh placentæ was divided into three parts and the following parallel experiments carried out:

Conditions of experiment	Result
A. Extract after free putrefaction	Strong pressor effect.
B. Extract after autolysis alone	Total absence of pressor effect.
C. Extract of fresh organ	Total absence of pressor effect.

[1] Bergell and Liepmann. *Münch. med. Woch.* No. 46, p. 2211. 1905. Cramer and Lochhead. *Proc. Phys. Soc.* p. xxiv. This *Journal*, xxxiv. 1906. Savarè. *Hofm. Beitr.* ix. p. 141. 1907.

Isolation and identification of the pressor principles.

Clear proof having been obtained that the formation of the pressor principle is due to the action of micro-organisms, further experiments showed that strict anærobic putrefaction is not essential and that prolonged putrefaction destroyed the active substances.

In order therefore to keep the putrefaction under control and as practical difficulties stood in the way of carrying out putrefactive processes on a large scale in this laboratory, it was thought advisable to return to the less offensive method of allowing partial putrefaction of the placentæ to proceed in the presence of small quantities of alcohol. Under these circumstances it seems that the formation of evil smelling products is largely, but not altogether, kept back and the active principle is not destroyed even after eight weeks.

The following procedure was finally adopted:

The minced fresh placentæ were mixed with absolute alcohol in quantities just sufficient to cover the material. 100—120 c.c. of absolute alcohol to 1 litre of the minced placentæ were usually taken. The mixture was allowed to stand at room temperature for some time, varying from 24 hours to several days. When ten litres had been collected, three litres of absolute alcohol were added, the mass strained through linen and pressed out in a hand-press. The filtrate was acidified with acetic acid and boiled up in order to coagulate the proteins. After filtration the clear yellowish extract was concentrated and absolute alcohol was added to the residue. The previous treatment was repeated until the residue was completely soluble in absolute alcohol.

From this extract the active principle was isolated by several methods. It was found that several pressor bases were present which were isolated as crystallised hydrochlorides.

One of these bases can be extracted from the alkaline solution by ether or by chloroform and is precipitated by basic lead acetate or mercury bichloride in alcoholic but not in aqueous solution.

Another base was obtained as a crystalline benzoyl product (by Baumann and Schotten's method) after removal of the other pressor bases from the alkaline solution by ether or chloroform. Although this substance had not been tested physiologically at the time of preparation, it was subsequently identified with the strong pressor base *p*-hydroxyphenylethylamine isolated by Barger and Walpole from putrid meat (see later). This base may or may not have been identical with a very powerful pressor substance which was obtained by precipitation with phosphotungstic acid, the quantity of which was, however, much less than that obtained by the other methods[1].

[1] The methods of isolation followed by me differed from those adopted by Abelous and his colleagues, as well as of those of Barger and Walpole, but in view of the exhaustive account of their methods given by the latter (see this *Journal*, *infra*, p. 344) it does not seem necessary to give further details.

The biphasic nature of the effect on blood-pressure always obtained by the injection of crude extracts (a fall of blood-pressure preceding the more marked and final rise) indicated the presence of a depressor as well as a pressor principle. As a matter of fact choline, the depressor action of which is well known, was found to be present in large quantities, and was isolated as a mercury salt. As much as 3·5 grammes of the latter were obtained from ten kilogrammes of placentæ. It was purified as a cadmium salt and analysed and identified as a platinum salt. Its physiological activity was demonstrated.

The presence of large quantities of tyrosine was also noticed amongst the products of putrefaction.

During the progress of this work, I became aware of the researches of Abelous and his collaborators, who had made the observation that the adrenaline percentage of suprarenal extracts was apparently increased by the addition of extracts prepared from various putrefied organs[1]. They found later that putrefied organs, and especially putrefied muscle, contained themselves a substance, different from adrenaline, which produced a rise of blood-pressure on intravenous injection[2]. It seemed apparent that the pressor substance, or substances formed during the putrefaction of meat, are identical with those produced under analogous conditions from placenta.

Abelous and Ribaut have since isolated and analysed a presssor base from putrid meat, to which they ascribe the formula $C_6H_{11}NO$[3]. Meanwhile G. Barger and G. S. Walpole had taken up the identification of the pressor principles of putrid meat, and kindly informed me of their results, which enabled me to identify the pressor bases, isolated by me from putrefied placenta, with those from putrid meat.

One of the pressor bases is identical with *p*-hydroxyphenylethylamine. It was identified by the fact that it gives Millon's reaction and by the melting point of its di-benzoyl derivative which is 169—170°. The melting point of the benzoyl product obtained by me from placenta was found to be 160° after preliminary purification. After five recrystallisations from alcohol, from which solvent it crystallised in needles, its melting point rose to 169°—170° and it was found identical with a sample of the synthetical product, with which I was able to compare it through the kindness of Dr Barger. As a further proof of identity I have found that a trace of the benzoyl product gives on

[1] *Compt. rend. Soc. Biol.* LIX. p. 589. 1905.
[2] *Ibid.* LX. pp. 463, 530. 1906.
[3] *Ibid.* LXI. p. 908. 1908.

boiling with formaldehyde and sulphuric acid an intense green colour reaction. This emerald green colour is given both by the product from placenta as well as by the artificial benzoyl derivative. A similar reaction has been described as characteristic for tyrosine by Dènigès[1] and by Mörner[2].

The second pressor base isolated from placenta by its solubility in ether and chloroform (and also from the mercury salt insoluble in alcohol and soluble in water) is evidently identical with the base obtained by a similar method by Abelous and Ribaut, which has since been identified as isoamylamine by Barger and Walpole. The small amount of material I had left from the physiological experiments was not sufficient for chemical identification.

With regard to the precursors of these bases it has been suggested by Barger and Walpole in the case of putrid meat (and proved by them for *p*-hydroxyphenylethylamine) that they are derived from the corresponding amino-acids (leucine and tyrosine), the cleavage products of proteins. The same is probably true in the case of putrid placenta. It is of some interest to point out that the autolytic ferments of the placenta are unable to split off the terminal carboxyl-group of the amino-acids, without the help of micro-organisms.

SUMMARY.

1. Fresh human placenta does not contain any pressor principle.

2. The pressor principles of placental extracts, prepared according to Dixon and Taylor, are products of initial putrefaction.

3. The autolytic enzymes of the placenta, without the help of micro-organisms, are unable to produce pressor substances.

4. Several pressor bases were isolated from putrefying placenta, the most active of which was identified as *p*-hydroxyphenylethylamine, whilst the identity of another with isoamylamine is most probable. These bases are identical with those found by Barger and Walpole in putrid meat.

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[1] *Compt. Rend.* CXXX. p. 583. 1900.

[2] *Zeitschr. f. physiol. Chem.* XXXVII. p. 86. 1902.