material to the patient's blood reduces not only the clotting time, but also normalizes the prothrombin consumption as measured by SOULIER's[5] procedure one hour after clotting. A similar normalizing effect is exerted when the factor is added to oxalated hemophilic plasma and clotting is initiated by recalcification. Though there is no doubt that our preparations possess appreciable antihemophilic activity, more rigorously quantitative tests will have to be employed to prove the identity of the isolated platelet co-factor with the antihemophilic principle. Such a research is in progress.

It is interesting to note that the platelet co-factor in the partially purified form precipitates between 0.4 and 0.5 ammonium sulfate saturation, and not around 0.33–0.36 saturation as in the case when the factor is isolated from plasma[3,6]. This could be explained in terms of co-precipitation of the factor with other plasma proteins or by assuming two manifestations of the factor.

Whether the platelet co-factor (antihemophilic principle?) will turn out to be a catalyst or a stoichiometric partner in the prothrombin-thrombin conversion, its amount will represent only a trace fraction of the totality of the plasma proteins. We feel that our method for isolating the factor can be included easily in the large-scale plasma fractionation schemes (alcohol, ether) as a starting operation. Indeed, in general, it may be worthwhile to consider the possibility of removing important trace proteins from plasma by adsorbents (such as *e.g.* the platelet co-factor by kaolin, an antithrombin by $Al(OH)_3$[7], etc.), and then start with the precipitation techniques for preparing larger fractions.

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EFFECT OF MELANOPHORE-DISPERSING HORMONE ("B") ON LACTATING MAMMARY GLAND SLICES *IN VITRO*

by

T. R. BRADLEY AND S. J. FOLLEY

National Institute for Research in Dairying, University of Reading (England)

AND

F. W. LANDGREBE AND G. M. MITCHELL

Welsh National School of Medicine, Cardiff (Wales)

If mammary gland slices from lactating rats are incubated in the Warburg apparatus with acetate + glucose dissolved in KREBS-bicarbonate-saline in equilibrium with 95% O_2 + 5% CO_2, the overall pressure curve (composite curve) has a positive slope because the R.Q. is greater than 1[1]. BALMAIN AND FOLLEY[2] showed that addition of prolactin to the medium increased the slope of the composite curve of mammary gland slices taken from rats in early lactation. We have confirmed this with eight prolactin preparations of potency varying from 11–22 i.u./mg and have shown that the effect can be obtained with tissue from rats at all stages of lactation but not in late pregnancy or after weaning. The response was also given by mammary gland slices from lactating mice but not from guinea pigs, rabbits or sheep in early lactation. Liver slices from lactating rats gave no response to prolactin. However, the threshold concentrations were always rather high, the lowest being about 25 μg/ml and moreover there was no relation between prolactin potency and ability to increase the slope of the composite curve of rat mammary slices. These facts suggested that the metabolic effect might be due to some contaminant in the prolactin.

This suggestion was borne out by an examination of purified preparations of other pituitary hormones for their effect on the respiratory exchange of rat mammary gland slices. All were tested at a concentration of 500 μg/ml. Of fourteen growth hormone preparations six were found to possess appreciable activity, and five out of nine ACTH preparations were active. Activity was also found in each of two preparations of thyrotrophin but not in a single purified preparation of FSH. As regards the posterior pituitary, two purified oxytocin preparations were tested and found inactive while activity was found in one purified pressor preparation and in a specimen of VAN DYKE's posterior lobe protein.

TABLE I

Rat	*Day of lactation*	*Net gas evolution as μl CO_2/100 mg moist tissue/2 h at 37°*	
		Control	*"B"*
			2.5 μg/ml
1	6	25	80
		47	79
2	7	78	90
		56	100
3	9	42	108
		65	140
4	9	117	146
		100	147
			1.25 μg/ml
5	6	87	111
		72	107
		79	104
		95	108

There remained the intermediate lobe. A number of "B" preparations made by the method of LANDGREBE AND MITCHELL[3] were tested as well as two from other sources and all proved active in lower concentrations than any other type of pituitary preparation tried. These preparations were all made from pig gland. Ox preparations, which differ biologically from pig preparations[3], will be tested in due course. Preparations assaying (toad melanophore test of LANDGREBE AND WARING[4]) 350 i.u./mg (oxytocin < 0.5 i.u./mg; vasopressin < 2.0 i.u./mg; 0.3 mg intravenously showed no milk-ejection activity in an anaesthetized goat) increased the slope of the composite curve of rat mammary tissue at a concentration of 10 μg/ml and a more recent highly-purified preparation assaying 950 i.u./mg[3] (oxytocin < 0.25 i.u./mg; vasopressin < 5.0 i.u./mg) was active down to about 1.25 μg/ml (see Table). These results suggest that "B" may be the active principle present in prolactin and other pituitary preparations capable of affecting the respiratory exchange of mammary tissue *in vitro* (it is well known that most commercial ACTH preparations are potent extracts of "B"), though the possibility that the effect is due to some unknown contaminant present in purified "B" cannot yet be excluded. Further, the nature of the metabolic response of the tissue to "B" resulting in an increased slope of the composite curve remains to be discovered. By analogy with the similar effect of insulin *in vitro* on the respiratory exchange of rat mammary slices[1] it might be due to an increased R.Q. resulting from accelerated lipogenesis[5]; on the other hand, an increase in the rate of glycolysis could bring about the same result.

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