No melanophores of any kind differentiated in treated explants from New Hampshire Red embryos, even though there were occasional cases in which red melanophores appeared in the corresponding control cultures. Similarly, all hormone-treated White Leghorn explants were entirely negative, although the controls contained the usual numbers of black melanophores which are short-lived though capable of synthesizing and depositing melanin granules. In the case of the Barred Rock, which possesses a relatively more viable pigment cell, the result varied with the age of the explanted tissue. When skin was taken from a 6-day embryo or younger, no differentiated melanophores were present at the time of explantation, and few or none of the melanoblasts developed pigment granules in the presence of the hormone. Skin from 7-day embryos or older already contained many black melanophores at the time of isolation. When it was grown in the corticosterone-plasma the melanophores which were present retracted their processes, rounded up, and died, and new ones were not differentiated to take their places. The reaction was not always complete; occasionally, expanded melanophores persisted in older explants or were newly differentiated in younger ones, but there were never many of them. Control cultures always contained great quantities of black, expanded melanophores.

The action of the cortical hormone, then, is apparently that of a suppressor of melanin formation, which causes degeneration of melanophores and inhibits the differentiation of new ones. This result may be of interest in view of the fact that clinically it is observed that Addison's disease is accompanied by a darkening of the skin as though the normal inhibitor of pigment formation had been removed with the destruction of the adrenal cortex.

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Activation of Pitressin by Acetic Acid.*

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In the process of development of a technic for the extraction of pitressin from tissues as a preliminary to its assay, the effect of acetic acid on pitressin *in vitro* was studied.

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TABLE I.
Activation of Pitressin by Acetic Acid.

Preparation (1)	Dose given, M.U. Treated (2)	Dose given, M.U. Control (3)	Dose assayed, M.U. Treated (4)	Dose assayed, M.U. Control (5)	Activation factor Col. 4/Col. 2 (6)	Activation factor Col. 4/Col. 5 (7)
M.U.						
150 boiled c* 50 cc 1% acetic acid	13.95	—	45	—	3.2	—
1000 " c 10 " " " "	17.12	16.16	30.5	12.0	1.7	2.5
400 refluxed 15 min c 25 cc 1% acetic acid	4.68	4.76	8.1	11.4	1.7	0.71
400 " " " " " " " " " "	4.52	4.56	9.6	5.4	2.1	1.8
400 " " " " " " " " " "	8.30	8.76	13.2	8.3	1.6	1.6
4000 " " " " 20 " " " "	8.52	8.20	11.7	7.4	1.4	1.6
4000 " " " " " " " " "		Pressor Assay			1.6	1.6
4000 " " " " " " " " "	8.00	7.98	15.3	9.0	1.9	1.7
5000 " 10 " " 25 " " " "		Pressor Assay			1.6	1.5
4000 " 15 " " " " " " "		" "			1.7	1.3
1300 mixed cold c 10 cc 1% acetic acid	13.57	13.10	9.0	10.8	0.66	0.83
400 refluxed 15 min c water	4.01	3.99	1.5	5.8	0.37	0.26
4000 " " " " .1 N HCl		Pressor Assay			0.5	0.35
4000 " " " " .15 N Sod. Acetate		" "			0.8	0.8

The antidiuretic assays (Burn)[3] were made on a newly calibrated group of rats and doses of treated pitressin were alternated with doses of untreated pitressin.

The pressor assays were made by intravenous injection into deeply chloretonized dogs of alternate similar doses of treated and untreated pitressin (Kamm).[4] The activation factor in these instances was obtained from the ratio of the maximum blood pressure rise obtained with the control and treated substances (Column 7), and checked by a ratio of the areas under the pressure curves (Column 6).

Columns (2) and (3) are in doses per rat. The paired doses are identical on a weight basis.

*c = with.

[3] Burn, J. H., *Quart. J. Pharm.*, 1931, **4**, 517.

[4] Kamm, O., *J. Am. Chem. Soc.*, 1928, **50**, 573.

Table I shows the result of the assays of a variety of preparations.† Note in every case that the pitressin which had been boiled with acetic acid appears to have a definitely more potent effect than untreated pitressin, but that the other treatments involved a loss of potency. Column 1 indicates that this activation is a result of boiling with acetic acid rather than of boiling, concentration or acetate ions alone. The close similarity of the activation as measured by the antidiuretic method and by the pressor method is to be noted, though this does not necessarily indicate that the two effects (antidiuretic and pressor) are due to the same substance.

The failure of hydrochloric acid to activate pitressin (confirming Dale and Dudley[1] and others) suggests that the mechanism is not a hydrolytic one. The fact that the effect is observed equally with subcutaneous injection in the one assay and with intravenous injection in the other precludes the likelihood of the activation depending upon variations in the rate of absorption.

Noble, Rinderknecht and Williams,[2] observing potentiation of pitressin solutions assayed by the antidiuretic technic after treatment with certain heavy metal acetates, concluded that the effect of these augmenting substances depended on changes in the rate of subcutaneous absorption. The present investigation indicates the possibility that the observed augmentation may have been due to the effect of acetic acid derived from hydrolysis of the heavy metal acetates on the pitressin rather than to the effects of the salts on the rate of subcutaneous absorption.

Since acetic acid, which is commonly employed in the extraction of tissues for assay of pitressin content, is able to potentiate pitressin to a varying degree, there is a possibility that misleading results might be obtained by the application of this method unless extreme care is used in the standardization of all details such as the concentration of reagent and duration of procedures.

Summary. Pitressin when boiled with acetic acid becomes more potent as shown by subcutaneous antidiuretic assay and intravenous pressor assay. These effects are not due to boiling, concentration, acidity, or the presence of acetate ions. The significance of these findings in connection with the preparation of acetic acid extracts of tissues for pitressin assay is discussed.

† These preparations were all made by dilution from pitressin generously given to us by Dr. Oliver Kamm of Parke Davis Laboratories to whom our thanks are extended.

1 Dale, H. H., and Dudley, H. W., *J. Pharm.*, 1921, **18**, 1.

2 Noble, Rinderknecht, and Williams, *J. Physiol.*, 1939, **96**, 293.