that the isolated pigmented part of the tumor is strikingly high in copper concentration strongly favors the theory that copper acts as a local catalyst in pigment formation, probably as part of an oxidative enzyme, possibly tyrosinase which has recently been demonstrated in melanotic tumors of mice.[26,27] Mammalian tyrosinase contains significant amounts of copper and can be inhibited by substances which combine with this metal.[28] Copper apparently also forms a metallo-organic complex with dopa[29] and may remain attached to the melanin molecule. In this connection it is of interest to note that the ash of octopus ink contains as much as 2% copper.[30]

We advance the theory that copper may promote pigmentation, not only by direct action on the substrate, but also indirectly by oxidizing sulfhydryl groups which inhibit pigmentation *in vitro*,[31] and probably also *in vivo*.[8,32,33] Greenstein *et al.* have indicated that melanin in mouse melanomas is attached to the rest of the pseudoglobulin molecule in the vicinity of sulfur containing amino acids.[12] Our finding that copper, too, is closely linked to the pigment suggests an interaction between copper and sulfhydryl compounds.

Summary 1. Autoxidation of dopa *in vitro* was more strongly catalyzed by cupric ions than by any of the other heavy metallic salts investigated. The order of catalytic activity was Cu>Co>Ni>Mn>Pb>Fe.

2. Black and grey hair of rabbits, guinea pigs and rats generally, but not always, contained more copper than white hair of the same individual. In guinea pigs, red-brown hair contained more iron than white hair.

3. Analysis of the separated layers of human skin showed a considerably higher copper content in the epidermis than in the corium.

4. Melanin from mouse melanomas had a copper content 4 to 13 times higher than the tumor from which it was prepared.

5. The theory is advanced that copper plays a role as a local catalyst in mammalian pigmentation.

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An Antidiuretic Substance in the Blood of Normal and Adrenalectomized Rats.*

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In adrenal insufficiency the diuretic response to water is greatly reduced or absent (reviewed by[1]). An attempt was made to see if this was due to the accumulation of anti-

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TABLE I.
Showing Mean Effect on Water Diuresis Produced by Various Types of Blood Serum.

Series	Type of serum tested: (1 cc sample)	No. animals	% water excreted at indicated min. 30	60	90 ± S.E.*	Urine chlorides mg/cc as NaCl	Total 3 hr
1	Normal—fresh	17	4	15	35 ± 3.56	1.06	11.15
2	Adx. 3-7 days—fresh	26	2	10	26 ± 2.22	1.14	11.82
3	Adx. 9-12 " "	10	1	6	20 ± 4.03	1.29	13.23
4	Normal—drawn 12 hr	16	20	45	66 ± 4.97	0.45	5.56
5	Adx.— " 12 "	5	9	32	51	0.55	7.07
6	Normal saline (no serum)	12	19	45	64 ± 3.67	0.43	6.21

* S.E. $= \pm \sqrt{\frac{\Sigma d^2}{n(n-1)}}$

diuretic substances (called ADS) in body fluids. The use of a modified Heller and Urban[2] technic revealed that the serum of normal animals contained a consistently detectable amount of labile ADS and that its concentration increased after adrenalectomy.

Methods. In testing samples of blood serum for their antidiuretic content, male rats weighing approximately 200 g were fasted but allowed water for 18 hours. Two doses of water, 3 cc per 100 sq cm of body surface, warmed to body temperature, were then administered at hourly intervals to animals in individual metabolism cages. For a 200 g rat each dose was 9 cc. One hour after the administration of the second dose of water the urine was measured. Any rat which, during the 2 hour hydration period, had a urine excretion rate 50% more or less than the mean of the groups of 6 was discarded. This necessitated the elimination of 6%. The rats with a normal diuretic response were then placed over clean funnels, the test material was injected intraperitoneally, and a third dose of water was administered. Following the third dose of water the urine output was measured at 30 minute intervals for 3 hours. The water excreted was calculated as the per cent of the total water given (3 doses) minus that excreted prior to injection of test material. It was found that this method, designed not so much to equalize hydration, as to establish a high diuretic rate, permitted better determinations of ADS than the ordinary Burn assay procedure. The most consistent differences between groups occurred at 90 minutes and figures beyond that time are not tabulated here.

To obtain serum, blood was taken directly from the heart without anticoagulant after induction of ether anesthesia. The blood was quickly centrifuged, serum decanted, and immediately injected into test animals unless otherwise specified.

Results. As seen in Table I, Ser. 1, the injection of 1 cc of serum from normal animals depressed urine volume and increased chloride excretion. If the decanted serum samples were allowed to stand at 5 - 9°C for 12 hours the antidiuretic and chloruretic activity was lost (Ser. 4). The serum of animals adrenalectomized from 3 to 7 days had higher antidiuretic and somewhat higher chloruretic activity than that of normal animals (Ser. 2). Again most of the activity was lost in aged refrigerated serum (Ser. 5). Greater amounts of ADS appeared in animals adrenalectomized for 9 - 12 days (Ser. 3). For the first 18 hours after adrenalectomy rats show a normal diuretic response to small (5 cc per 100 g) water loads but not to larger ones.[1] It was found (Table II) that 5 milliunits of Pitressin† was more effective at 18 hours after adrenalectomy than in dummy-operated controls.

Discussion. While antidiuretic substances have been detected in body fluids in various conditions, results have been in general either

2 Heller, H., and Urban, F. F., *J. Physiol.*, 1935, **85**, 502.

† Supplied by Dr. D. A. McGinty, Parke, Davis and Company.

TABLE II.
Water Excretion in Rats Receiving Water (5 cc/100 g) and 5 Milliunits Pitressin.

	No. animals	% excreted at following min. 60	120	180 ± S.E.
Dummy-operated	12	5.5	47.5	79.9 ± 3.52
Adx. 18 hr	12	4.1	27.6	55.0 ± 4.50

scattered, inconsistent or controversial.[3-6] The test used here apparently reveals the presence of ADS consistently in normal rat serum. This method has been used by Dr. C. W. Lloyd of Syracuse University Medical College and yielded similar results (unpublished) both in normal human subjects and Addisonian patients. While our data is consistent with the hypothesis that the circulating ADS is of posterior pituitary origin, critical evidence on its site of origin and precise quantitative information regarding its activity are being sought.

Although it is obvious that the greater accumulation of ADS may account in part for the failure of water diuresis in adrenalectomized animals, a factor of equal importance may be the increased sensitivity to—rather than the increased amounts of—ADS. This is indicated by the observed augmented sensitivity to Pitressin after adrenalectomy. Thus, normal water excretion may be a consequence of the balanced interaction of diuretic cortical hormones and ADS of pituitary or other origin (concept of Corey and Britton[7]). After adrenalectomy, the ADS, present in increased amounts, could act unchecked by its normal antagonist. Such interpretations are consistent with recent studies on renal function in salt-treated adrenalectomized rats, which indicate that the failure of water excretion is due primarily to an accelerated tubular reabsorption (Lotspeich[8]). Although a decreased glomerular filtration may be observed in untreated adrenalectomized rats, it will not account for the failure of water excretion at moderately high water loads.[9] If the main factor involved is an active ADS, possibly of posterior pituitary origin, its expected action would be on renal tubular reabsorption. The problem, however, is a complex one because extra-renal factors have also been clearly demonstrated.[1,10]

Summary. The blood serum of normal rats contains a labile antidiuretic and chloruretic substance(s?) which increases in amount after adrenalectomy.

Addendum. Since the above was written additional work, in collaboration with Drs. W. R. Boss and C. M. Osborn, has established that: (1) the ADS in fresh normal rat serum does not affect glomerular filtration, as measured by creatinine clearance, and presumably therefore acts by increasing the renal tubular reabsorption of water; and (2) that no ADS is detectable in the blood of hypophysectomized rats.

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[8] Lotspeich, W. D., *Fed. Proc.*, 1948, **7**, 74; and additional data by personal communication.

[9] Unpublished work of Drs. W. R. Boss and J. H. Birnie of this laboratory.

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