

Table 2. DISTRIBUTION OF NICOTINAMIDE DEAMIDASE IN THE SUBCELLULAR COMPONENTS OF THE LIVER AND THE KIDNEY OF THE PIGEON

The incubation system (2 ml.), buffered at pH 7.5, contained nicotineamide (2.5 mgm.) and cell fraction equivalent to 50 mgm. of fresh tissue. Incubation at 37° C. for 120 min. in the case of liver and 90 min. in the case of kidney

Fraction	Liver			Kidney		
	Nicotinic acid released ( $\mu$ gm.)	Per cent total activity	Nicotinic acid released per mgm. nitrogen* ( $\mu$ gm.)	Nicotinic acid released ( $\mu$ gm.)	Per cent total activity	Nicotinic acid released per mgm. nitrogen* ( $\mu$ gm.)
Unfractionated homogenate	252.0	100.0	192.3	570.0	100.0	422.3
Nuclei	162.3	64.3	417.6	141.0	24.7	363.4
Mitochondria	33.0	32.9	389.6	334.0	58.6	1421.0
Microsomes	43.0	17.1	201.8	70.5	12.4	298.7
Supernatant	21.4	8.3	36.5	45.0	7.9	78.9

\* Nitrogen present in enzyme preparation, and insoluble in trichloroacetic acid, determined by Nesslerization of a sulphuric acid-hydrogen peroxide digest.

The highly selective distribution of nicotinamide deamidase, evident from the fact that most of the vertebrates examined, the avian species excepted, do not possess the enzyme and that in the chick, whereas the liver is completely devoid of the enzyme, the kidney has it in abundance, would point to the specific nature of the enzyme as against its being a peptidase or similar enzyme acting non-specifically.

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### Chromatographic Detection of Minute Quantities of Thyroxine in Serum

EFFORTS to detect extracted iodized compounds directly on chromatograms have been made by various authors while following the metabolism of thyroid hormones. Nevertheless, because of low sensitivity of the agents currently employed (ninhydrin, diazotized sulphanic acid) this has proved fruitless. By modifying the original Sandell-Kolthoff reaction for iodine estimation, Bowden *et al.*<sup>1</sup> succeeded in detecting 0.1 $\gamma$  of thyroxine. The detection of thyroxine following chromatography of serum required the use of large amounts of the biological material<sup>2</sup>. Moreover, careful purification of the extract for chromatography was laborious and time-consuming. Efforts made so far<sup>1,3</sup> have likewise failed to bring about the desired results because the colour spots are of short duration.

By means of a minor modification of the procedure of Bowden *et al.* we have succeeded in detecting thyroxine in 1.0 ml. of serum. The chromatogram was sprayed with a solution (1:1) of ceric ammonium sulphate (0.2 N) - sodium arsenite (1.0 N) and left approximately 15 min. to dry at room temperature after a preliminary superficial desiccation. It was then passed through a 0.5 per cent solution of fluorescein in acetone, or a 5.0 per cent solution of anthranilic acid in acetone. When the chromatogram had dried in a free current of air, thyroxine was detected by means of ultra-violet light. For a visual localization of thyroxine the chromatogram was gently sprayed with a 1.0 per cent solution of *o*-toluidine in acetone instead of the fluorescein solution. Spots, pale yellow in colour, appeared on a background of dark brown. These spots remain stable for a considerable period of time and the colour contrast increases with time. The sensitivity attained by these processings is of the order of 0.01 $\gamma$  of thyroxine.

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### Nature of the Carcinogenic Substance in Egg-Yolks

A HIGH incidence of malignant tumours was recently reported in mice fed egg-yolks<sup>1</sup>, and the presence of a growth-promoting factor in the yolks has been thought to be responsible for this effect<sup>2</sup>. This substance was found to be soluble in common fat solvents and it is contained in the non-saponifiable and non-phospholipid portion of the yolk<sup>3</sup>.

In the course of my investigations concerned with the isolation of the tumour-enhancing factor, I have prepared a lipid substance accelerating the growth of experimental tumours in animals as well as stimulating the incorporation of labelled methionine-<sup>35</sup>S into the proteins of Ehrlich ascites carcinoma *in vitro*. Only traces of this material are present in tissues of normal mice and rats when compared with organs of tumour-bearing animals.

The active substance is readily extracted from the unsaponifiable portion of fresh tissues or frozen-dried organs by benzene, acetone or ether. It is not adsorbed on alumina columns, but retained to some extent on anion-exchange resins, from which it can be displaced by means of weak acids. A paper chromatographic procedure has been applied for the isolation of the factor from eluants after ion-exchange chromatography.

The pure material is readily soluble in water and common fat solvents, giving an intensive yellowish fluorescence and characteristic ultra-violet absorption spectra. It is labile to heat (90° C.), destroyed by repeated freezing and thawing, but not by freeze-drying. No detailed chemical analyses could so far be carried out of a very small quantity of active material which could be recovered from tissues of tumour-bearing rats. The properties of this material so far examined suggest its close relation to aromatic hydrocarbons, possibly bile acids. It is definitely