Anti-beta-lipoprotein antibody titres

Titre	0, 1/2	1/4, 1/8	1/16	1/32	1/64	Positive (%)	Positive (number)
Controls	55	5	0	0	0	0	0
Diabetes	115	44	12	7	2	11	21/180
Nephritis	52	5	1	2	0	5	3/60
Tuberculosis	18	60	9	1	0	11	10/88
Amyloid	-	-	-	-	2		

with pulmonary tuberculosis had a raised antibody titre of $^1/_{16}$ or higher. Even at a titre as low as $^1/_{4.8}$ the patients with diabetes or tuberculosis differed from control and nephritis patients.

Discussion. The fact that this antibody is present only in low titre may explain why it has not been previously noted. There is an extensive literature on LDL polymorphisms[7], mainly based on patients with very high haemagglutination titres following multiple transfusions. LDL antigens AG(a), Ag(c), Ag(e) are inherited as autosomal dominants. Such antibodies are normally rare and occur in less than 1% of healthy blood donors. The antibody described here occurred in low titre in patients with either juvenile or maturity onset diabetes. The finding might be relevant to the inheritance of diabetes but it is more likely that this antibody occurs as a phenomenon secondary to the development of atherosclerosis.

The LDL antigen that has been used might be heterogenous. The finding of a similar antibody in pulmonary tuberculosis and amyloid suggests that the antigen could be cross-related to mycobacterial lipid antigen[8]. Similar lipid has been used in a haemagglutination reaction for detection of tuberculosis[9].

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Estimation of the methylating capacity of the pineal gland. With special reference to indole metabolism

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Summary. In order to obtain more information on the methylating capacity of the pineal gland, a method determining the formation of different 5-methoxyindoles in the pineal gland was developed. The method depends on measuring the incorporation of labelled methyl groups into the various hydroxyindoles present in the pineal gland, after incorporation of pineal tissue with labelled S-adenosyl methionine. Hydroxyindoles were not added to the incubation medium. After incubation thin-layer chromatography was performed with pineal tissue together with the incubation medium; the spots were scraped and counted.

The development of a HIOMT assay by Axelrod and Weissbach[2] offered the possibility of obtaining information about the methylation of N-acetylserotonin[2] or other 5-hydroxyindoles[3], depending on the particular 5-hydroxyindoles added as a substrate. However, no account is taken of the possibility that there are several more-or-less specific HIOMT enzymes[3-5] which are involved in the synthesis of the different 5-methoxyindoles (melatonin, 5-methoxytryptophol, 5-methoxytryptamine, 5-methoxyindole-3-acetic acid and 5-methoxytryptophan). To obtain more information about the methylating capacity of the pineal gland, with special reference to the indole metabolism, a new method was developed. 1 rat pineal was excised, slightly disrupted and incubated at 37 °C with 20 µl 0.1 M of phosphate buffer (pH 8.0) and 10 µl S-adenosyl methionine-^{3}H (1.5 µCi/10 µl) in H_2SO_4 (pH 2.5). The 5-hydroxyindoles present in the pineal were used as a substrate without addition of N-acetylserotonin or any other 5-hydroxyindole to the incubation medium. After incubation for 60 min at 37 °C, the reaction was stopped with 10 µl H_2SO_4 (pH 1.0). Stopping the reaction with a sodium borate buffer (pH 10.0) was avoided because in alkaline medium S-adenosyl methionine (the methyl donor) shows a rapid desintegration (figure 1). These decomposition products cause complications if TLC is applied. The incubated pineal tissue is then homogenized in the incubation medium and the 5 above-mentioned synthetic 5-methoxyindoles (1 µg of each) are added for reference purposes. Without concentrating, the pineal tissue together with the incubation medium and the synthetic 5-methoxyindoles were chromatographed by TLC (Merck DC-silicagel plates 60F254, 0.25 mm No. 5729 were used).

Chromatograms are developed in darkness (to minimize decomposition) in chloroform:methanol:ammonia (25%), 60:35:5[6]. Separation was obtained between 5-methoxytryptophan, 5-methoxyindole-3-acetic acid, 5-methoxytryptamine and localized in 1 spot: melatonin and 5-methoxytryptophol. Identification of the spots of the 5-methoxyindoles was facilitated by the previous addition of the synthetic compounds. The latter show a fluorescence when the thin-layer plate is examined under an UV-light source (λ_{max} 254 nm). To identify the tritiated products, the plate was

examined with a thin-layer scanner (figure 2) (Berthold Dünnschicht Scanner II LB 2723). Parallel to the pineal tissue S-adenosyl methionine, which was manipulated similarly, was chromatographed with and without tissue of the cerebral cortex, to identify tritiated decomposition products and to check the influence of brain tissue on R_f values. The spots were then scraped from the thin-layer plate, placed in counting vials with 75 µl of ethanol and 10 ml of a scintillation liquid (toluene 1000 ml; POPOP 0.1 g; PPO 5 g; and for dispersion of the silica gel Cab-o-sil 40 g) and counted (liquid scintillation counter Mark I of Nuclear, Chicago). The tritiated decomposition products of S-adenosyl methionine, localized on the identical R_f values of the 5-methoxyindoles, are subtracted from the activity of the 5-methoxyindole spots of the examined pineal tissue. The results are demonstrated in figure 3. Research is in progress to separate the spot of melatonin and 5-methoxytryptophol into its components.

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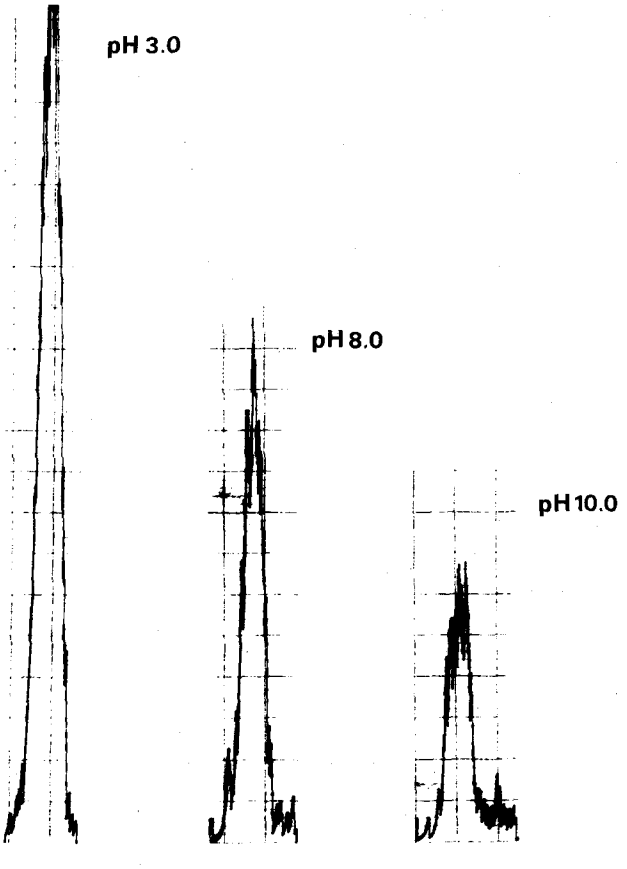

Fig. 1. Radioactivity of S-adenosyl methionine-^{3}H (remaining on the start) after stopping the reaction at different pH values.

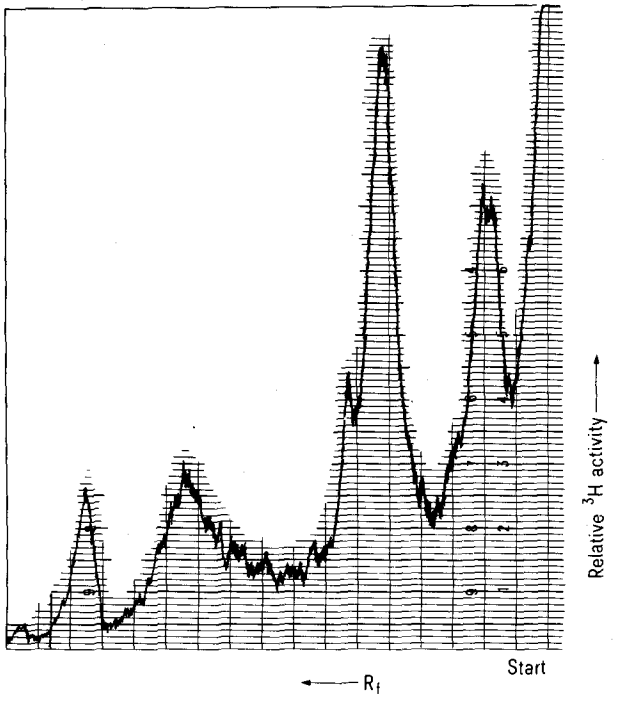

Fig. 2. Results of scanning of radioactivity of one pineal incubated with S-adenosyl methionine-^{3}H showing tritiated methylated compounds together with the tritiated decomposition products.

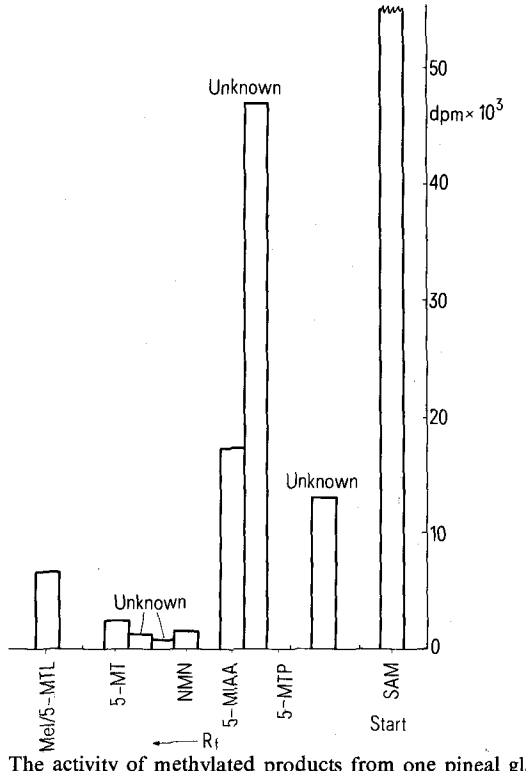

Fig. 3. The activity of methylated products from one pineal gland obtained by subtracting the activity of the decomposition products of S-adenosyl methionine from the radioactivity demonstrated in figure 2. The R_f values of the added methoxyindoles are indicated. The identity of some methylated products remains unknown.
Mel, melatonin; 5-MTL, 5-methoxytryptophol; 5-MT, 5-methoxytryptamine; 5-MTP, 5-methoxytryptophan; 5-MIAA, 5-methoxyindole-3-acetic acid; NMN, normetanephrine; SAM, S-adenosyl methionine.