fibrinogen was then obtained by recording the increase in the turbidity of fibrinogen polymerization by nephelometry at 370 nm according to the method of Michael et al.[12]. To elucidate further the polysaccharide nature of the ASP, it was treated with 0.01 M KIO_4 in tris-hydrochloride buffer, pH 8.4, at 27 °C for 5, 10, 15 and 20 h. Heat stability of the ASP was tested by maintaining at 120 °C for 10 and 30 min in tris-hydrochloride buffer, pH 8.4. For the examination of the effect of different pHs on its biological stability, it was dissolved in phosphate buffer at pH 6.0, 8.0 or 10.0. These were determined by the gel formation time as mentioned above.

Results. Normal gel formation time of fibrinogen mixed with intact ASP alone was 90 sec whereas it was 150, 230 and 330 sec when it was pretreated with glucose oxidase, α-galactosidase and β-N-acetylglucosaminidase, respectively. However, no gel formation was observed after treatment of ASP with galactose oxidase, β-galactosidase or mixed glucosidases. The increase of turbidity for fibrinogen polymerization matched the gel formation time as shown in figure 1. With treatment by periodate oxidation for 5 h, the ASP prolonged the gel formation capacity. With the same treatment for 20 h, gel formation was completely destroyed. When the ASP was heated at 120 °C for 10 min, gel formation time was prolonged to 320 sec; however, this activity was completely destroyed by heating for 30 min. Gel formation was lost below pH 6.0, but was quite stable between pH 8.0 and 10.0 as shown in figure 2. Further, similar results were observed when prothrombin-free plasma was used in place of fibrinogen.

Discussion. In a previous paper[11] it was noted that the ASP, designating as the CCFAS, was capable of reacting either with fibrinogen or fibrinogen degradation products resulting compact-colony formation of *S. aureus* strains in fibrinogen or serum-soft agar. The substance was totally different from protein A, coagulase and clumping factor, as mentioned above, and contained neither sialic acid nor any common enzymes including the proteolytic enzymes. Thus the ASP was considered to be the 3rd staphylococcal substance related to blood clotting in addition to coagulase and the clumping factor.

In these experiments, the ASP obtained from *S. epidermidis* was sensitive in its biological activity to both galactose oxidase and β-galactosidase, but was resistant to β-N-acetylglucosaminidase. These properties suggest that the digestion of the galactose moiety in the ASP could be caused by 2 former enzymes. Also, it was suggested that the ASP may convert fibrinogen to fibrin by direct action. In this case, the carbohydrate moiety of this substance, especially galactose, would be closely related to fibrinogen polymerization in an alkaline condition. This alkaline stability of the ASP would represent a biological advantage in normal alkaline tissue fluid. Further investigation concerning the relationship between fibrinogen polymerization by the ASP and other blood clotting substances are in progress in our laboratory.

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An antibody to low density lipoprotein in diabetics

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Summary. The serum of 21/180 unselected diabetics contains a low titre antibody to human low density lipoprotein. A similar antibody was found in 10/88 persons with tuberculosis.

Iso-antibodies against low density lipoprotein (LDL) or high density lipoprotein (HDL) are found in patients who have been transfused with blood or plasma repeatedly[1], in occasional persons with myeloma[2], and as a cause of hypolipidaemia in rheumatoid arthritis and cancer[3]. Some years ago Beaumont, Jacotot and Beaumont[4] found antibodies to LDL in patients with advanced ischaemic heart disease. Although they then suggested that the antibody might be a cause of atherosclerosis, the finding could be a secondary phenomenon. This report concerns the finding of anti-LDL antibodies in patients with either diabetes mellitus or pulmonary tuberculosis.

Methods. Sera were collected from unselected patients with diabetes, pulmonary tuberculosis or nephritis. Specimens sent to the biochemical laboratory were used as controls. The sera were decomplemented by heating at 55 °C for 30 min and were then adsorbed with sheep red cells. Agglutination tests were performed with sheep red cells coated with chromic chloride[5] to which LDL antigen was attached. This LDL was obtained from human serum by manganese chloride-heparin precipitation[6]. After dialysis separation of the LDL was achieved by ultra-centrifugation in a sodium chloride density tube at 105,000 × g for 18 h. In this way lipoproteins Sf 0–12 were obtained for use as antigen. The preparation was Australia antigen negative.

Results. These are shown in the table according to the haemagglutination titres using doubling dilutions of serum. 40 µg of antigen when added to 0.3 ml serum was found to reduce the antibody titre from $\frac{1}{8}$ to $\frac{1}{2}$.

It will be noted that 11% of diabetics and 11% of patients

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