Table 1. R_F values and color reactions with DMBA of amiben, amiben-X from soybean, and *N*-glycosyl amiben; DMBA being *p*-dimethylaminobenzaldehyde in a mixture of equal parts of methanol and HCl. Y, yellow.

R_F with solvent system			Color with DMBA	
I	II	III	0.01*N* HCl	0.5*N* HCl
		Amiben		
0.45–.55	0.30–.45	0.67–.73	Y	Y
		Amiben-X		
.23–.27	.03–.05	.63–.73		Y
		Amiben-X after acidic hydrolysis		
.45–.55	.30–.45	.67–.73	Y	Y
		N-*glycosl amiben*		
.23–.27	.03–.05	.63–.73		Y

to the culture medium. Five days later soybean roots were harvested, washed, and extracted with methanol. Extracts were concentrated and chromatographed on Whatman 3 MM paper. Chromatograms were developed by the ascending method in a mixture of *n*-butanol, ethanol, and ammonium hydroxide (2 : 1 : 1 by volume) (system I); of *n*-butanol, ammonium hydroxide, and water (8 : 1 : 1 by volume) (system II); or of phenol, water, and ammonium hydroxide (75 : 21 : 4 by volume) (system III). Amiben and its conjugate were located on the chromatograms by spraying them with *p*-dimethylaminobenzaldehyde (DMBA) in a mixture of methanol and 1*N* HCl (1 : 1). Other reagents sprayed were ninhydrin to detect amino acids and *p*-anisidine to detect sugars (*3*). The *N*-glycosyl derivative of amiben was prepared in the manner of Haugaard and Tumerman (*4*) for *N*-glycosyl-*p*-aminobenzoic acid.

The first step was to determine whether amiben-X prepared from soybean was in fact a conjugate or some other type of metabolite, such as a degradation product. Acidic hydrolysis of the soybean extract in 0.5*N* HCl (15 to 20 minutes at room temperature) resulted in complete release of amiben from amiben-X, indicating that amiben-X was a conjugate (Table 1). Furthermore, it appeared that amiben-X and *N*-glycosyl amiben were chromatographically identical (Table 1). Acidic treatment of pure amiben resulted in no change in chromatographic behavior.

The next step was to establish the point of attachment of amiben in the conjugate. One might expect that the amino and carboxyl groups of amiben would be the most susceptible points of attack. Since the spray reagent DMBA reacts with aromatic amino groupings, a lack of color response by amiben-X would indicate that the amino grouping of amiben was not free. When chromatograms were sprayed with DMBA in 0.01*N* HCl, amiben gave a yellow color but amiben-X and *N*-glycosyl amiben gave no response (Table 1). However, when DMBA was sprayed in 0.5*N* HCl all three compounds responded with a yellow color. Amiben responded rapidly; but, with amiben-X and *N*-glycosyl amiben, development was delayed, indicating that hydrolysis of these compounds occurred before color development was possible. This similarity in response to DMBA of amiben-X and *N*-glycosyl amiben is further evidence that they are the same compound.

Both compounds were also practically insoluble in ether and *n*-butanol, relatively insoluble in acetone, but quite soluble in methanol or water; both were stable at temperatures as high as 65°C but relatively unstable above 100°C. They were unstable at *p*H 3.0 or lower, but relatively stable at *p*H 7 to 12. Moreover, amiben-X, before or after hydrolysis, did not respond to ninhydrin, an indication that amino acids or proteins were not implicated. Finally, amiben-X seemed to be associated with sugar, since it responded to *p*-anisidine. It was concluded that amiben-X is *N*-glycosyl amiben. The sugar moiety of amiben-X is probably glucose but more evidence is needed to establish this.

The physiological significance of amiben-X was determined by chromatographing extracts of resistant (soybean) and susceptible (barley, *Hordeum vulgare*) plants after their exposure to amiben-C^{14} (1.1 mc/mmole). Seeds of both species were incubated for 3 days at 30°C in amiben-C^{14} (45 ppm). They were placed in petri dishes on filter papers containing the herbicide solution. This concentration of amiben-C^{14} inhibited root elongation about 50 percent in soybean and more than 90 percent in barley. Methanol extracts of the seedlings were chromatographed; the extraction with methanol removed over 90 percent of the radioactivity present.

Essentially all the radioactivity extracted from soybean was present as a compound that corresponded to *N*-glycosyl amiben. In barley, however, about 15 percent of the radioactivity corresponded to *N*-glycosyl amiben, about 35 percent corresponded to free amiben, and the remaining activity was in two unidentified compounds that moved close to the solvent front when system I was used. These compounds are also conjugates in that they released amiben. Thus it appears likely that the formation of *N*-glycosyl amiben is a mechanism for the detoxification of amiben in soybean.

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16 June 1965

Lithocholic Acid in Human-Blood Serum

Abstract. *Lithocholic acid was present in the serum of three patients with jaundice and, in smaller amounts, in two healthy adults. This bile acid occurs naturally in human feces but induces cirrhosis of the liver when fed to a wide variety of experimental animals. The finding of lithocholic acid in blood is of interest because of its possible role in injuring human liver.*

Lithocholic acid (3α-hydroxy-5β-cholanoic acid), first isolated from cattle gallstones (*1*) and normally present in human feces (*2*), produces cirrhosis of the liver when fed to animals (*3*). It is also a potent inflammatory agent, pyrogen, and hemolysin in man (*4*). Identification of this acid in human blood is of considerable interest because of the possibility that it may induce or abet liver injury or cirrhosis, or both, in man as it does in animals. Of possible significance is the observation that chenodeoxycholic acid (3α, 7α-dihydroxy-5β-cholanoic acid), the immediate precursor of lithocholic acid, is often the predominant bile acid in the blood of patients with severe liver disease (*5*). Lithocholic acid is formed in the colon by bacteria that remove the 7α-hydroxyl group from chenodeoxycholic acid (*6*).

Lithocholic acid was identified in human serum by gas-liquid chromatography. A mixture of 30 ml of serum and 270 ml of ethanol was heated to

boiling, cooled, and filtered. The filtrate was evaporated on a steam bath under nitrogen, and the residue was taken up in 30-percent aqueous ethanol adjusted to *p*H 11 with NaOH, and extracted with 100 ml of ethyl ether. The aqueous phase was dried by evaporation, and the residue was dissolved in 30 ml of 4.5*N* NaOH and heated in a stainless steel bomb for 24 hours at 130°C. The hydrolyzed material was diluted with an equal volume of water, the *p*H was adjusted to less than 1 with 12*N* HCl, and the material was then extracted twice with equal volumes of chloroform and once with an equal volume of ethyl ether. The chloroform-ether phase was evaporated under nitrogen, and the residue was either chromatographed on Celite columns (*7*) or methylated with diazomethane in methanol; it was then placed on a neutral alumina column (5 g dried for 18 hours at 130°C) and eluted with 40-ml volumes each of benzene, 10 percent ethyl acetate in benzene, 60 percent ethyl acetate in benzene, and finally methanol. Approximately 33 percent of the 1 : 10 ethyl acetate-benzene fraction (or the petroleum ether fraction from the Celite columns after methylation) was dried by evaporation, dissolved in acetone, and delivered to a 5-mm by 1.8-m glass column containing gas chrom Z (100 to 120 mesh) coated with 0.5 percent QF-1 (a silicone material) (*8*). Operating conditions were: column temperature, 220°C; argon flow, 62 ml/min; temperature of argon-ionization detector, 235°C.

The result is compared in Fig. 1 with a sample of authentic methyl lithocholate. The retention times (S to L) for peak L in serum and methyl lithocholate are identical (8.86 minutes). A second portion of the 1 : 10 ethyl acetate–benzene fraction was dried by evaporation, and the trifluoroacetate derivatives of the methyl esters were prepared by treating the residue with 0.2 ml of trifluoroacetic anhydride for 15 minutes at 30°C. The residue from the evaporated solution was taken up in acetone and run with the same column and under the same conditions as those used for the methyl esters. The results (Fig. 2) show that the methyl trifluoroacetoxy derivatives of lithocholic acid in serum (peak L) has the same retention time (S to L) as the authentic methyl 3α-trifluoroacetoxy-5β-cholanate shown for comparison (8.03 minutes).

By this means, lithocholic acid was shown to exist in the blood serum of three jaundiced patients and, in smaller amounts, in two normal subjects. Table 1 shows the diagnoses, supported by liver biopsy of each patient, with concentrations of bilirubin and bile acids in serums. The method used to measure trihydroxy and dihydroxy bile acids in serum does not measure monohydroxy lithocholic acid (*5*).

If losses of lithocholic acid during hydrolysis are approximately uniform, gross estimates of the relative amounts of lithocholic acid in the normal and jaundiced serums studied would show about two to five times as much in the jaundiced group. The increase is correlated in a general way with the observed increase in concentrations of trihydroxy and dihydroxy bile acids in serums.

In the two healthy subjects, additional evidence for the identity of lithocholic acid in the serum was obtained by subjecting the 1 : 10 ethyl acetate–benzene fraction from the alumina column or the petroleum ether fraction from the Celite columns after methylation to thin-layer chromatography (benzene and acetone at 7 : 3, on silica-gel, ascending, 25 minutes) (*9*). The zone opposite the control lithocholic acid spot (determined by exposure to iodine vapor) was removed, eluted with acetone, and subjected to gas chromatography. The methyl lithocholate peak again showed the same retention time (8.86 minutes) as authentic methyl lithocholate.

The method employed in this preliminary search for lithocholic acid in

Table 1. Diagnoses (in italics) and concentrations of bilirubin and bile acids in serums of three jaundiced patients and of two normal subjects.

Bilirubin (mg/100 ml)		Bile acids (μg/ml)	
1 minute	Total	Trihydroxy	Dihydroxy
	Biliary cirrhosis		
24.8	40.0	43.5	34.8
	Chronic hepatitis		
2.9	5.7	3.6	36.4
	Metastatic carcinoma		
10.1	20.8	42.4	37.0
	Normal		
0.1	0.3	0	0.8
.2	.7	1.0	0

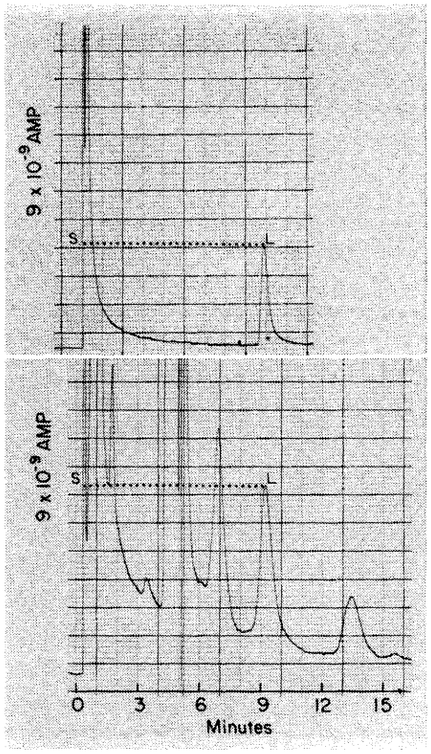

Fig. 1. Gas-liquid chromatography of extract of human serum (lower tracing), showing methyl lithocholate (peak *L*) having same retention time (*S* to *L*, 8.86 minutes) as that of authentic methyl lithocholate (upper tracing).

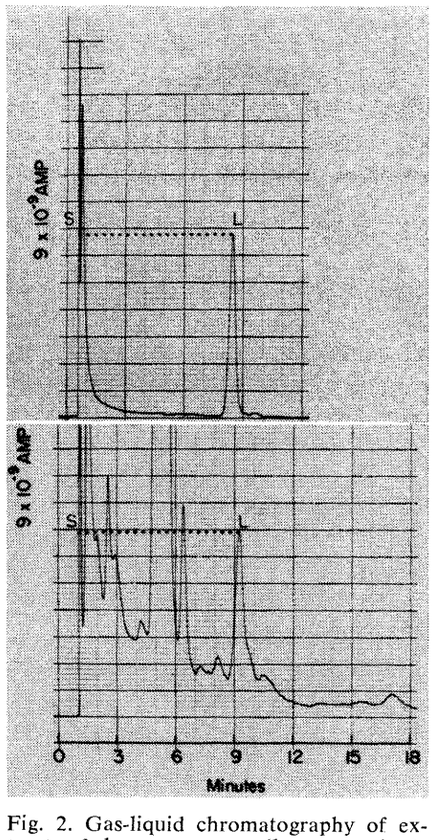

Fig. 2. Gas-liquid chromatography of extract of human serum (lower tracing), showing methyl trifluoroacetoxy lithocholanate (peak *L*) having the same retention time (*S* to *L*, 8.03 minutes) as that of authentic methyl trifluoroacetoxy lithocholanate (upper tracing).

serum cannot be regarded as quantitative because the conditions used to hydrolyze taurolithocholic acid lead to partial destruction or alteration of the lithocholic acid molecule. Evidence of this was obtained by hydrolyzing taurolithocholic acid-24-C^{14} under the same conditions and noting the appearance of several widely distributed radioactive peaks under Celite-column chromatography. Use of milder conditions normally sufficient to hydrolyze conjugates of dihydroxy and trihydroxy bile acids or use of 50 percent aqueous ethanol resulted in incomplete hydrolysis of taurolithocholic acid.

It is apparent from this study, however, that enough lithocholic acid survived hydrolysis to be detected by gas chromatography, as was demonstrated by the appearance of both the methyl ester and trifluoroacetate derivatives of lithocholic acid in the serum extracts. Using a technique employing extraction by anion-exchange resin, Sandberg *et al.* (*10*) observed a peak, believed to be lithocholic acid, in their gas-chromatographic analysis of serum bile acids from a patient with hepatitis. Sufficient data to permit direct comparison with our studies were not given; their study was primarily concerned with other bile acids in serum.

The finding of lithocholic acid in the serums of patients with liver disease raises important questions regarding the possible role of this compound in injury to the human liver. Relatively little is known, for example, about the amount of lithocholic acid absorbed from the colon; quantitative studies are needed to determine whether amounts absorbed are comparable with those required in animal-feeding experiments to produce injury to the liver. Lithocholic acid is believed to be poorly absorbed from the colon because very little or none is found in human bile (*11*) and it remains in the sediment of fecal extracts (*6*). Its presence in serum, however, raises the possibility that more lithocholic acid is absorbed from the colon than is generally recognized.

Another question concerns the toxicity of lithocholic acid to the human liver. Lithocholic acid has produced proliferation of hepatic ductular cells and other changes in the liver in birds (chicken, *12*), reptiles (iguana, *13*), and mammals (rats, *14*; rabbits, *3*; mice, *14*; and monkeys, *15*). It is likely that man also is subject to its cirrhotogenic effects, and perhaps he is more sensitive than the rat (*14*), since the human liver does not readily hydroxylate lithocholic acid to other compounds (*16*), as do the livers of Muridae (*17*).

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18. Supported by PHS grant A-713.

17 June 1965

Template Activity of RNA from Antibody-Producing Tissues

Abstract. *An RNA fraction, which represents a small percentage of cellular RNA and which has the characteristics of nuclear messenger RNA, has been isolated from the spleen and lymph nodes of immunized rats by successive phenol extractions of these tissues at increasing temperatures. This fraction increased the amount of protein synthesized in a cell-free extract of* Escherichia coli *as much as 35 times and directed the synthesis of proteins different from those of* E. coli.

According to the "instructive" theories of antibody formation, the specificity of antibodies results from the presence, at the site of γ-globulin synthesis, of the antigen itself, which somehow determines the tertiary (*1*) or even the primary (*2*) structure characteristic of a specific antibody; the role of the genetic information is, in this model, limited to the formation of unspecific γ-globulin molecules. In contrast, the "selective" theories postulate that the specificity of all possible antibody molecules is determined by the genome itself (*3*); each of a multitude of different genes would be potentially capable of coding for that small portion of the γ-globulin molecule of which the unique amino acid sequence accounts for the specificity of a given antibody.

Since genetic information is expressed in the form of mRNA (*4*), the contribution of the genome in the control of antibody specificity could be assessed by determining whether or not mRNA of antibody-producing cells is capable, in the absence of antigen, of directing the synthesis of specific antibody molecules. We report now the first step in such a study, the isolation from antibody-synthesizing tissues of an RNA fraction with the characteristics of mRNA and, particularly, with a high template activity, defined as the ability to stimulate the synthesis of proteins.

RNA was extracted from spleen and lymph nodes of rats killed at various times after the injection, into the peritoneal cavity and the four footpads, of a mixture of *Haemophilus pertussis* and sheep red blood cells. Whole organs or subcellular fractions were homogenized in a mixture of equal volumes of buffer (0.1*M* tris, *p*H 5, with 0.5 percent naphthalene disulfonate) and 88 percent phenol; RNA was purified by repeated extractions with phenol and precipitations with ethanol (*5*). The capacity to stimulate the incorporation of amino acids into protein in a cell-free system derived from *Escherichia coli* was taken as a measure of the template activity of the RNA preparations. RNA obtained from animals which were in the primary or in the secondary phase of the immune response gave similar results and therefore will not be discussed separately.

RNA extracted from whole organs