Table 2. Capacitation of sperm in Locke's solution (phosphate-buffered) and in uterine fluid (UF) in vitro.

Sperm incubation		Time of insemination*	No. of ova	No. of ova cleaved
Environment	Hr			
Locke's	6	10.5	22	3
Locke's	6	12.0	33	2
Locke's	8	10.5	32	0
Locke's	8	12.0	29	0
UF	6	10.5	95	40
UF	6	12.0	47	1
UF	8	10.5	29	15
UF	8	12.0	32	2
UF, dialy.†	6	10.5	51	0
UF, macro.†	6	10.5	48	8
UF, decap‡	7	10.5	17	0

* Tubal inseminations; hours after injection of LH. † Dialysate (dialy.) and macromolecular (macro.) portions of uterine fluid. ‡ Sperm incubated in uterine fluid and decapacitated with seminal plasma.

tor. Previous research from our laboratory had demonstrated a rabbit uterine fluid component which electrophoretically and immunochemically resembled beta-globulin (*9*). These observations suggested to us that reactant(s) in uterine fluid similar to amylase may be normally involved in sperm capacitation. Consequently, we assayed (*10*) for amylase activity and found that 1 ml of rabbit uterine fluid liberated an average of 0.15 mg of maltose equivalents from starch. The comparable average for blood serum was 0.93.

Preliminary data (Table 3) revealed that sperm incubated in beta-amylase (1.0 or 0.1 mg/100 ml of phosphate-buffered Locke's solution) apparently were capacitated. A total of 114 ova were recovered, of which 40 were cleaved following tubal inseminations, about 0 to 1.5 hours after ovulation, with sperm which had been incubated for 8, 10, or 12 hours in beta-amylase. The control data for these experiments are those resulting from sperm incubations with phosphate-buffered Locke's solution (Table 2). Eight does were similarly inseminated with sperm incubated with beta-amylase, and two of them possessed fetuses at 12 days of gestation. The fertility data (Table 3) from sperm incubations with beta-amylase supported the view that partial capacitation resulted from the beta-amylase and that capacitation was completed in the oviducts of the tubally inseminated does. However, the possibility that longer periods of incubation with beta-amylase might completely capacitate sperm could not be excluded on the basis of these data.

Table 3. Capacitation of sperm with beta-amylase.

Sperm incubation		Time of insemination†	No. of ova	No. of ova cleaved
β-amylase* (mg%)	Hr			
1.0	8	10.5	11	3
0.1	8	10.5	13	5
.1	8	12.0	18	4
.1	10	10.5	18	8
.1	10	12.0	17	5
.1	12	10.5	21	10
.1	12	12.0	16	5
		Total	114	40

* Beta-amylase in phosphate-buffered Locke's solution. † Tubal inseminations; hours after injection of LH.

Sperm were incubated at 38° to 39°C in all of these experiments. Motility was always observed before and after treatment of the sperm. Incubation *in utero* or in uterine fluid in vitro always resulted in a reduction of the percentage of motile sperm, from 60 to 70 percent initially to 20 to 50 percent at the end of the incubation periods. Of washed sperm and sperm incubated in buffered Locke's solution, with or without beta-amylase, at least 50 percent were motile at the time of tubal inseminations. Thus, the differences in fertility apparent in Tables 1, 2, and 3 were not attributable to differences in sperm motility.

Bedford and Chang (*11*) observed that the seminal plasma decapacitation factor was a macromolecule and proposed a hypothesis which was recently modified (*12*) to suggest that capacitation may involve destruction or removal of seminal plasma factors on sperm. Weil and Rodenburg (*13*) demonstrated that rabbit sperm are coated with seminal plasma antigens, and Hunter and Hafs (*14*) demonstrated that some seminal antigens firmly coat bull sperm before the secretions of the major accessory sex glands come in contact with the sperm. These observations are compatible with data which indicated that epididymal as well as washed ejaculated sperm must be capacitated to fertilize ova (*1*).

To our knowledge, the data reported in this paper provide the first demonstration of in vitro capacitation of sperm. Furthermore, the data suggest that sperm capacitation involves enzymatic alteration of carbohydrate-containing macromolecules from seminal plasma which coat sperm and inhibit fertilization.

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Herbicide Metabolism: N-Glycoside of Amiben Isolated from Soybean Plants

Abstract. *The* N-*glycoside of the herbicide 3-amino-2,5-dichlorobenzoic acid (amiben) was synthesized in the laboratory. The compound was chromatographically identical with a conjugate of amiben isolated from soybean plants,* Glycine Max *(L.) Merr. The seeds of a tolerant plant, soybean, and of a susceptible one, barley* (Hordeum vulgare *L.), were incubated in amiben labeled with carbon-14, and the seedlings were extracted. Essentially all the radioactivity in soybean was present as a compound corresponding to* N-*glycosyl amiben. Only a small amount of this conjugate was present in barley; most of the radioactivity was present as free amiben or as other unidentified metabolites.*

Conjugates of the herbicide 3-amino-2,5-dichlorobenzoic acid (amiben) have been isolated from plants. Their structure was not reported, but acidic or alkaline hydrolysis of conjugates from tomato plants (*1*) and from soybean plants (*2*) released free amiben. I now report on a conjugate of amiben in soybean plants, amiben-X, and on its possible physiological significance.

Soybean plants were grown in sand culture in pots in the greenhouse. When the plants were 10 to 12 days old, amiben (1 mg per plant) was added

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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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