Biochemical Effects of Sucrose Acetate Isobutyrate (SAIB) on the Liver[1] (37074)

B. G. Procter, P. Dussault, and C. I. Chappel
Bio-Research Laboratories Ltd., Pointe Claire, Quebec, Canada

Sucrose acetate isobutyrate (SAIB) is employed as a flavor-suspending agent in the manufacture of soft drinks. Presently, Canadian regulations permit its use at a level of 50 ppm in conjunction with 15 ppm of brominated vegetable oils. To estimate its safety for use at higher levels, the effects of SAIB were investigated in feeding studies involving rats and dogs. Liver function parameters, in particular, were investigated in these experiments and in separate acute studies involving rats and primates.

Materials and Methods. In the beagle dog, SAIB was fed for 12 wk at dietary levels of 0, 0.5, 1, 2 and 4% for Groups I to V, respectively. Group VI dogs were fed 2% SAIB for 12 wk and a standard ration for 6 wk prior to slaughter. At intervals during the experiment, serum samples were obtained from all animals and were tested for glutamic pyruvic transaminase (1), glutamic oxalacetic transaminase (2), alkaline phosphatase (3), lactic dehydrogenase (4), bilirubin (5) and ornithine carbamoyl transferase (6). In addition, clearance of bromsulfophthalein (BSP) from the plasma was assessed (7). Plasma clearance of indocyanine green (ICG) was determined, prior to the conclusion of the study, in the untreated males and in the Group V males fed SAIB at the 4% level. The method of Bonasch and Cornelius (8) was employed.

In a second feeding experiment, the effects of SAIB on liver function were investigated in albino rats of the Sprague-Dawley strain. The study consisted of two populations of rats treated for 6 and 12 wk respectively, and the feeding levels of SAIB employed were 2.5, 5 and 10%. Serum samples obtained at the end of the experiment were analyzed for glutamic pyruvic transaminase, alkaline phosphatase, ornithine carbamoyl transferase and bilirubin.

Separate acute experiments were undertaken to investigate the effect of SAIB on the clearance of BSP from the plasma of the squirrel monkey (*Saimiri sciureus*) and from that of the albino rat.

In the case of the squirrel monkey, two groups of three male animals, each weighing approximately 1 kg, were employed. Animals of Group I were treated with SAIB following an overnight fast and, 24 hr after treatment, they were tested for BSP clearance. Treatment consisted of the administration by stomach tube to each monkey of 2 g of SAIB suspended in 4 ml of cottonseed oil. Simultaneously, plasma BSP clearance was studied in the untreated subjects of Group II. Seven days later, BSP clearance of all monkeys was again tested. On this occasion, however, Group I animals remained untreated while each of those in Group II was treated with 2 g of SAIB in cottonseed oil 24 hr prior to the liver function test.

Three groups of five male albino rats were fed a ration containing 4% SAIB *ad lib.* for 7 days. Testing of BSP plasma clearance in these three groups was carried out 0, 24 and 48 hr following termination of feeding for Groups II to IV, respectively. Group I subjects received no treatment whatsoever prior to testing for BSP plasma clearance whereas each Group V animal (positive control) was tested 24 hr following a single ip injection of carbon tetrachloride at a dose level of 1 ml/kg (9).

Results. 1. Beagles. Increased BSP retention occurred among all animals of both sexes of all treatment groups at each of 4, 8 and 12 wk of SAIB administration. Group mean re-

[1] This investigation was supported entirely by The Canadian Soft Drink Association.

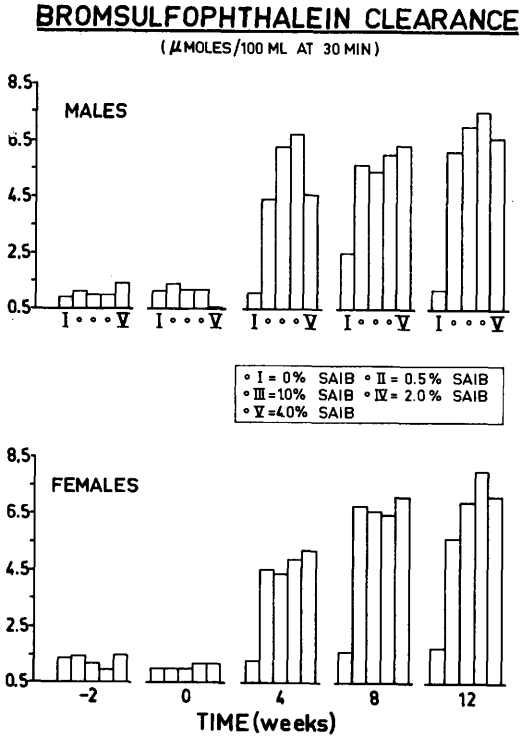

FIG. 1. Impairment of bromsulfophthalein plasma clearance in beagles during feeding of SAIB at various dietary levels.

sults (μmoles/100 ml at 30 min) are illustrated in Fig. 1 for animals of both sexes and it is evident that the effect of treatment with SAIB on BSP clearance was uniformly great at all treatment levels. Despite this profound effect of treatment with SAIB on BSP clearance, other blood biochemical parameters, with the following exception, remained within normal limits. Increases (Fig. 2) in levels of serum alkaline phosphatase (SAP) were directly related to duration of SAIB administration as well as to dosage level. Examination of dogs (Group VI) fed a standard ration for 6 wk following 12 wk feeding of SAIB at a dietary level of 2% demonstrated the complete reversibility (Fig. 3) of the effects of this treatment on BSP clearance and on SAP. In addition, the animals of Group V were fed a standard diet for 3 wk following conclusion of 12 wk treatment with SAIB (4%). During this time the rate of clearance of BSP from the plasma of these animals gradually returned to normal.

Four naive beagle dogs were fed a single meal containing 4% SAIB and the effect of this treatment on plasma BSP clearance and on levels of serum bilirubin and SAP was investigated (10). Within 18 hr of SAIB administration, all four subjects responded with increased plasma BSP retention but subsequent testing proved that this effect was fully reversed within 48 hr. Serum bilirubin and SAP levels, moreover, remained normal. Additional studies with SAIB in two naive dogs were directed at plasma BSP clearance alone. Again, a meal containing 4% SAIB (18 g) was fed to each dog and both subjects responded with impaired plasma BSP clearance as early as 1 hr after SAIB administration.

Finally, plasma clearance of ICG was determined for the control and high dose males at the conclusion of the 12-wk feeding period. Displayed in Fig. 4, the results of the ICG clearance testing suggest an impairment of liver excretory activity and are thus in agreement with the results of testing for plasma clearance of BSP.

2. Albino rats. During 12 wk of feeding of SAIB to albino rats at dietary levels as high as 10%, blood biochemical studies in-

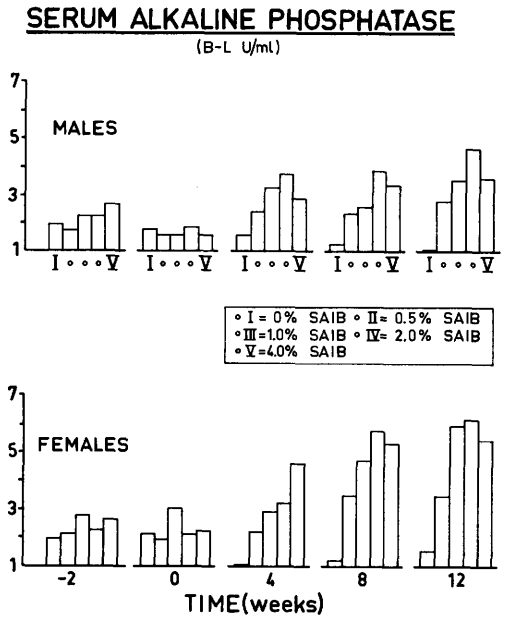

FIG. 2. Elevation of serum alkaline phosphatase levels in beagles during feeding of SAIB at various dietary levels.

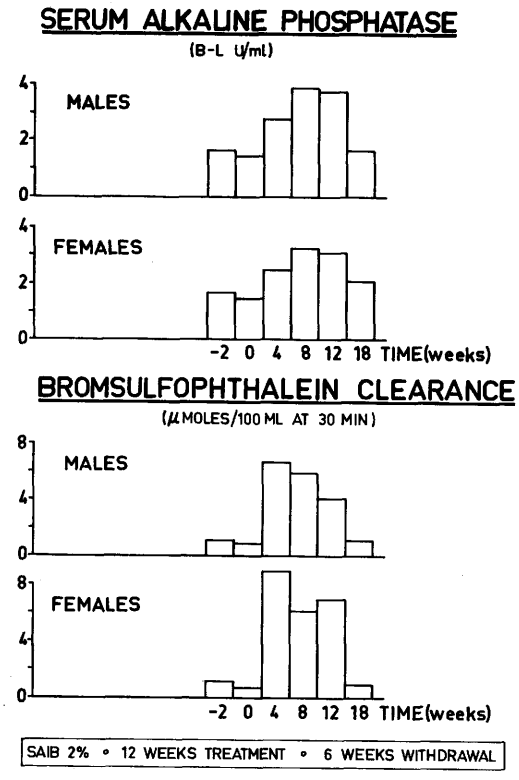

FIG. 3. Reversibility of the effects on serum alkaline phosphatase levels and on bromsulfophthalein plasma clearance in beagles fed SAIB at a dietary level of 2% for 12 wk.

dicated the absence of any effect of this compound on liver function. Plasma BSP clearance was not one of the parameters assessed.

3. Acute experiments. Since plasma BSP clearance could not be assessed in the rat feeding experiment discussed above, this parameter was studied in separate groups of rats fed SAIB (4%) *ad lib.* for 4 days. The results, displayed in Table I, point out the absence of any effect of treatment with SAIB on the rate of clearance of BSP from the plasma of the rat. There was a similar absence of effect of SAIB on clearance of BSP from the plasma of the squirrel monkey following massive single treatments (Table II).

Discussion. Feeding studies with SAIB in beagles, rats and squirrel monkeys revealed effects associated with this compound only in the beagle. The primary effect was one of mild cholestasis as shown by impaired BSP clearance from the plasma and by increased levels of SAP. This cholestasis was revealed under the electron microscope by changes in biliary morphology (11) and also, in histochemical studies, by increased enzyme activity of the bile canaliculi (10). Specifically, the electron microscopic studies revealed slight dilatation of the bile canaliculi and an increase in the number of bile pigment granules. In the histochemistry studies, high activity of the bile canaliculi of SAIB-treated animals was revealed in the determinations of alkaline phosphatase, adenosine triphosphatase and glucose-6-phosphate dehydrogenase.

The second effect of feeding SAIB to beagles was liver hypertrophy, seen in the male subjects, and accompanied by proliferation of smooth endoplasmic reticulum, by hypertrophy of individual hepatocytes and by increased carboxylesterase activity (11). These latter changes were likely related to induction of metabolic enzymes and thus might represent adaptation to overloading. Similarly, it is postulated that the mild cholestasis described above is related to reversible interference by high levels of SAIB in biliary excretory mechanisms. Both effects, in any event, were quickly reversed by SAIB

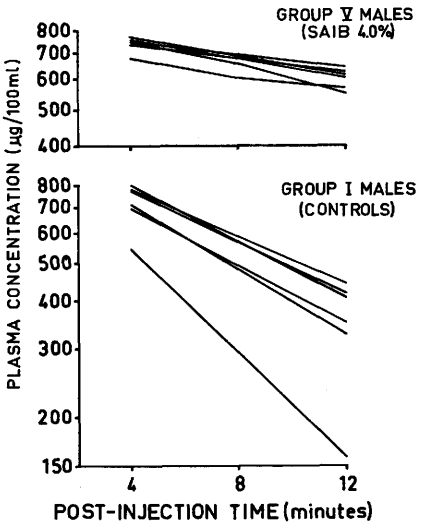

FIG. 4. Impairment of indocyanine green plasma clearance in beagles following feeding of SAIB at a dietary level of 4% for 12 wk.

TABLE I. Bromsulfophthalein Clearance in the Albino Rat.[a]

Group no.	Rat no.	Posttreatment (hr)	Plasma bromsulfophthalein (μmoles/100 ml)
	101		0.50
	102		0.65
I	103	Untreated	0.53
	104	controls	0.84
	105		0.44
	201		0.65
	202		0.48
II	203	0	0.72
	204		0.94
	205		0.46
	301		0.58
	302		0.51
III	303	24	0.62
	304		0.52
	305		0.90
	401		0.86
	402		0.44
IV	403	48	0.37
	404		0.53
	405		0.22
	501	Positive controls	1.21
	502	(BSP clearance testing	1.72
V	503	24 hr following single	1.61
	504	ip injection of 1 ml/kg	1.19
	505	CCl_4)	1.37

[a] SAIB-treated subjects (Groups II, III, and IV) were allowed free access, for 7 days, to a standard laboratory rodent diet containing 4% SAIB.

withdrawal and were unaccompanied by histotoxic changes (11).

In the case of the rat, SAIB proved to have no effects, toxic or otherwise, when fed at levels as high as 10% and for as long as 12 wk. In particular, SAIB produced none of the changes, biochemical or morphological, in the liver of the rat that it produced in the liver of the beagle dog (11).

Feeding of SAIB to beagle dogs in this research had a novel effect on clearance of BSP from the plasma of treated subjects. Impaired clearance of this dye and of ICG occurred not only during long-term administration of SAIB but, in the case of BSP at least, it also occurred as early as 1 hr after ingestion of a single meal containing 4% SAIB. In the acute studies, furthermore, plasma clearance rates for BSP returned to normal levels within approximately 48 hr and, even after 12 wk administration of SAIB (4%), values for this parameter returned to normal within 3 wk. The rapid onset and speedy reversibility of this effect of SAIB on BSP clearance in the dog suggest that it is a physiological or functional alteration rather than a manifestation of a toxic change. Since this work suggests that the effect of SAIB on the hepatic excretory mechanisms is peculiar to the dog, SAIB may therefore serve as a useful tool in studies of liver function.

Summary. During 12-wk feeding studies in rats and beagles, the effect of SAIB on liver function was investigated. Mild cholestasis and liver hypertrophy developed only in the dog and these effects were fully reversible and unaccompanied by histotoxic changes. Impaired clearance of BSP from the plasma oc-

TABLE II. Bromsulfophthalein Clearance in the Squirrel Monkey.

Monkey no.	Treatment	Plasma bromsulfophthalein (μmoles/100 ml) 24 hr posttreatment
101		0.49
102	SAIB, 2 g	0.84
103		0.45
201		4.12
202	None	0.68
203		0.49
	7 Day rest period	
101		6.40
102	None	7.58
103		0.82
201		0.38
202	SAIB, 2 g	0.54
203		0.61

curred in beagles but not in rats or squirrel monkeys treated with SAIB.

1. Wroblewski, F., and LaDue, J. S., Proc. Soc. Exp. Biol. Med. **91**, 569 (1956) as modified by Henry, R. J., Chiamori, N., Golub, O. J., and Berkman, S., Amer. J. Clin. Pathol. **34**, 381 (1960).

2. Karmen, A., J. Clin. Invest. **34**, 131 (1955) as modified by Henry, R. J., Chiamori, N., Golub, O. J., and Berkman, S., Amer. J. Clin. Pathol. **34**, 381 (1960).

3. Bessey, O. A., Lowry, O. H., and Brock, M. J., J. Biol. Chem. **164**, 321 (1946).

4. Henry, R. J., Chiamori, N., Golub, O. J., and Berkman, S., Amer. J. Clin. Pathol. **34**, 381 (1960).

5. Malloy, H. T., and Evelyn, K. A., J. Biol. Chem. **119**, 481 (1937).

6. Brown, R. W., and Grisolia, S., J. Lab. Clin. Med. **54**, 617 (1959).

7. Poutsiaka, J. W., Keysser, C. H., Thomas, B. G. H., and Linegar, C. R., Toxicol. Appl. Pharmacol. **4**, 55 (1962).

8. Bonasch, H., and Cornelius, C. E., Amer. J. Vet. Res. **25**, 103 (1964).

9. Klaassen, C. D., and Plaa, G. L., Toxicol. Appl. Pharmacol. **12**, 132 (1968).

10. Dussault, P., Procter, B. G., and Chappel, C. I., Abstr. No. 253, Ann. Meet. Can. Fed. Biol. Soc., 14th, Toronto, 1971.

11. Procter, B. G., Dussault, P., Rona, G., and Chappel, C. I., Abstr. No. 143, Ann. Meet. Soc. Toxicol., 11th, Williamsburg, 1972.

Received Oct. 5, 1972. P.S.E.B.M., 1973, Vol. 142.