In certain circumstances, the antigenic stimulus to RF production appears evident. It may appear in significant amounts in the presence of septicemia; when the infection clears, RF activity decreases(6). But if rheumatoid factor is an antibody, why does it persist in high titer in the serum of patients with rheumatoid arthritis in the absence of a definite antigen? If one rejects the concept of humoral hyperreactivity, then the possibility of a continuous, potent antigenic stimulus might be favored.

Humoral antibody hyperreactivity to Brucella antigen was not apparent in these patients with rheumatoid arthritis. The degree of their reactivity to other antigens not yet studied is not predictable; furthermore, no information was obtained regarding the possibility of hyperreactivity of a cellular nature.

Summary. No relationship was found between the antibody response to Brucella antigen and the F II hemagglutination titer in a series of patients with rheumatoid arthritis and osteoarthritis. Those with rheumatoid arthritis showed no greater antibody response to inoculation with Brucella antigen than did those with osteoarthritis. These observations do not confirm the report of antibody hyperreactivity in rheumatoid arthritis.

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Effect of Physical Factors on Liver Amylase Activity.* (28557)

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Other investigators(1,2) have reported that amylase is bound to particulate matter in tissue extracts and that the extracts must be subjected to additional treatment other than just normal homogenization if full measurement of amylase activity is to be obtained. Brosemer and Rutter(1) found that their rat liver homogenates and various liver fractions, particularly the microsomes, must be treated with Triton X-100 or subjected to sonication in order to demonstrate maximal amylase activity of such preparations. Grabowski and Munro(2) found that amylase activity of microsomes from pigeon pancreas was maximal only after incubation of microsomes with certain amino acids or after disintegration of microsomes with ballotini beads. Zalkin *et al.* (3) showed that inclusion of 0.2% Triton X-100 in the lysosome preparation from rabbit muscle was necessary completely to release ribonuclease, cathepsin, β-galactosidase and aryl sulfatase, and Wattiaux and deDuve (4) found that exposure of rat liver mitochondria to 0.1% Triton X-100 releases the same bound hydrolases.

To test whether conditions for eliciting maximal amylase activity of our liver preparations have been used in previous work, some of these methods mentioned above have been applied under the conditions prevailing in our experiments.

Methods. Livers were obtained fresh from white rats and dogs sacrificed under nembutal anesthesia in the laboratory. Hog liver was obtained fresh from the killing floor of the abbatoir. For preparation of homogenates from unfrozen liver, portions were removed

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and the remainder of the livers frozen overnight at −18°C.

Liver homogenates were prepared in the Waring blendor at a 1:50 dilution with cold (5°C) 0.9% NaCl for the dog and hog liver; 1:100 dilution for rat liver. In these experiments, the Waring blendor was carefully and thoroughly washed to remove any traces of detergents that might have been used in previous washings. All glassware was likewise thoroughly washed without detergent, rinsed with concentrated nitric acid and then finally with water. Portions of homogenates were subjected to the full power of a 10-Kc Raytheon sonicator for one minute, to other portions were added Triton X-100 (a liquid nonionic detergent) in amounts ranging from 0.1% to 1%. These procedures were carried out both on fresh, unfrozen portions of liver and also on portions which had been frozen and thawed.

Amylase activities of the liver homogenates and sera were determined by Van Loon's(5) method following the procedure previously described(6).

Using the methods previously described(7, 8), the effects of rabbit, rooster and goat antisera to hog pancreatic amylase on the activities of hog liver amylase and rat liver amylase were also determined on these homogenates subjected to sonication and detergent. The 1:20 dilutions of the antisera in each case were those previously determined to give the maximal inhibition (Table III) of hog pancreatic or rat pancreatic amylase but which gave little or no inhibition of various liver amylases.

Results. In experiments in which the amylase activity of a homogenate of fresh liver was compared to that of a homogenate of a portion of the same liver which had been frozen and rethawed, it was seen that the amylase activity was increased somewhat by the freezing (Table I) process for rat and hog liver but not dog liver. For rat liver this agrees with our previous report(6) as well as that of Brosemer and Rutter(1). Rat liver amylase activity was increased 52% and hog liver amylase activity increased 28%. We did not, however, find any increase in the amylase activity of the liver homogenates subjected to sonication (treatment with ultrasonic vibration) and the detergent, Triton X-100 (Table II). It was determined that amylase activities of the liver homogenates were not changed by sonication with treatment times up to one minute, the treatment time reported by Brosemer and Rutter(1).

TABLE I. Effect of Freezing and Thawing on Liver Amylase Activity.

	Amylase activity (units/100 g)		
Liver from	Control	After freezing and thawing	Ratio
Rat (5)	2150	3270	1.52
Hog (3)	453	625	1.38
Dog (3)	1010	1070	1.06

Numbers in parentheses indicate No. of livers used.

TABLE II. Effect of Triton X-100 and Sonication on Liver Amylase Activity.

	Amylase activity (units/100 g)			
Liver from	Control	With 0.1% Triton X-100	Sonicated	Ratio treated control
Rat (4)	2450	2550		1.04
(2)	2790	2590*		.93
(2)	2360		2560	1.08
Hog (3)	750	710		.95
(3)	770		810	1.05
Dog (2)	1040	1020		.98

* 1% Triton X-100. At this concentration of detergent the starch-iodine color was green rather than blue.

In the experiments testing the effects of sonication and detergent on the lack of inhibition of liver amylases by antisera to pancreatic amylase, no significant differences were noted between the results on ordinary liver homogenates and those obtained with homogenates subjected to sonication or detergent (Table III).

Discussion. As mentioned earlier, other investigators have shown that in some cases, various tissue extracts or tissue fractions must be subjected to more vigorous treatment than simple homogenization if one wishes to measure the complete enzyme activity of the tissue. We have shown that freezing and thawing does increase amylase activities of rat and dog liver but, in contrast to the findings of others, subjecting rat, hog and dog liver homogenates to concentrations of Triton X-100 of 0.1% and 1% did

TABLE III. Effect of Detergent and Sonication on Amylase Antisera Action on Liver Amylase Activity.

	Antisera to hog pancreatic amylase from								
	Rabbit* (1:20 dilution)			Goat† (1:20 dilution)			Rooster‡ (1:20 dilution)		
Liver amylase	Control	Triton X-100	Sonication	Control	Triton X-100	Sonication	Control	Triton X-100	Sonication
	% inhibition of amylase activity								
Hog	12	15		11	1				
				8	0				
				7		11			
				12		6			
Rat							2	5	
							0		0

* This antiserum inhibited hog pancreatic amylase 90%.
† " " " " " " 85%.
‡ " " " rat " " 63%.

not increase their amylase activities. Likewise, sonication did not cause any appreciable increase in amylase activities of these livers. Conchie *et al.*(9) have indicated that β-glucuronidase activities of pig liver, kidney and spleen were not increased by Triton X-100. We cannot explain why our results differ from others but are convinced that the differences are real. In any event, since the livers used in our experiments are routinely frozen and thawed before homogenates for amylase determinations are prepared, we feel that our previous results(6,10) are valid even though neither detergent treatment nor sonication was used.

The Van Loon method of amylase determination uses a starch concentration of 0.04% in the reaction mixture and consequently requires more dilute tissue extracts than other methods which may use up to 2% starch substrate. Perhaps in more dilute solutions, amylase is more readily released from its bound form than at higher starch and tissue concentrations.

The lack of inhibition of the amylase activities of liver extracts by various antisera to hog pancreatic amylase has been interpreted in this laboratory as evidence that liver amylase is structurally distinct from pancreatic and salivary amylases(7,8). In the present investigation we have shown that treatment with detergent or sonication does not affect this lack of inhibition and the possibility that the lack of inhibition was merely due to inability of the amylase antibodies to react with bound liver amylase is therefore disproved. In any event, one would have to assume a condition in which the starch molecules could react with the bound amylase whereas the antibodies could not. Furthermore, if one uses a liver extract from which all particles have been removed by centrifugation (100,000 *g* for 30 minutes) and which would contain only soluble amylase, its amylase activity is also not inhibited by amylase antisera.

Summary. The measurable amylase activities of rat and hog livers are increased by freezing and thawing but are not further enhanced by sonication or treatment with the detergent Triton X-100. Therefore, values for liver amylase activities previously reported from this laboratory are not low as has been suggested. The lack of inhibition of liver amylases by antisera to pancreatic amylase is not due to binding of liver amylase to particulates.

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Effect of Anabolic Steroids on Liver Function Tests in Rabbits (28558)

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An increasing number of reports of abnormal liver function tests following administration of certain drugs have appeared in the recent literature(1,2). Noteworthy among the compounds that produce these changes are the anabolic steroids(3,4,5). The two compounds most extensively studied have been methyltestosterone and norethandrolone (17γ-ethyl-19-nortestosterone). These compounds not infrequently cause an increased bromsulfalein (BSP) retention and less frequently increased serum transaminase activity. Published studies have shown that most of these abnormalities disappear following withdrawal or even with continued administration of the drug(5,6). Increases in BSP retention and serum transaminase activity have not been reported in animal studies following administration of drugs which have been shown to cause these changes in man.

Although Popper and Schaffner(7) detected changes in the microvilli of some bile canaliculi in human and rat liver by electron microscopy following norethandrolone administration, Gass and Umberger(8) reported daily administration of 10-100 mg of methyltestosterone and norethandrolone for periods up to 50 days had no effect on BSP retention in dogs. Recently Clodi *et al.*(9) and Kolb *et al.*(10) published reports regarding the effect of anabolic steroid administration on elimination of certain organic dyes in the bile of rats. Clodi and associates showed an initial decrease (0-45 minute interval) followed by an increase (45-90 minute interval) in BSP elimination in rat bile after 7-day administration of 6 mg/kg/day of methyltestosterone. Kolb and associates measured the effect of 8 different anabolic steroids on bile elimination of I-131 labeled rose bengal in bile-fistula rats. Seven of the 8 steroids including methyltestosterone and norethandrolone, administered in doses of 5 mg/kg/day, caused a retardation in bile elimination of varying significance on first and/or 7th and/or 14th test day, but none on 21st test day.

This report concerns experiments undertaken to determine the effect of methyltestosterone and norethandrolone administration on liver function in rabbits as measured by serum glutamic-oxolacetic transaminase activity (SGOT) and disappearance of BSP from the blood stream.

Material and methods. Male and female young white New Zealand rabbits weighing 2.5-3.5 kg were given Purina rabbit chow and water *ad libitum*. The compounds were prepared for injection in polyethylene glycol 200 (PEG), 25 mg/ml and administered IM daily—10 mg/kg of body weight for 6 days. Control animals were injected with an equivalent amount of PEG in the same manner. Percent BSP retention was measured prior to compound administration and on the 6th test day by a modified procedure especially designed for rabbits. Twenty mg of dye per kg of body weight was injected into marginal ear vein. A blood sample for assay was obtained by cardiac puncture exactly 20 minutes later using a #23 gauge one-inch needle and a 2 cc syringe wet with heparin to prevent clotting. To minimize the effect of lipemia associated with anabolic steroid administration, the BSP was assayed according to the procedure of Henry *et al.*(11). Blood for SGOT assay was drawn from marginal ear vein prior to injection of dye in each experiment and was measured by the method of Karmen *et al.*(12). It was neces-