types of localized antibody referred to by Nossal. The possibility that localized hemolytic antibody may persist in lymphatic nodules as a result of primary immunization and that such antibody is detectable by the FST may allow one to predict the readiness of the immune mechanism to experience a secondary response.

Summary. The kinetics of the hemolytic antibody response in mice to a single injection of sheep erythrocytes were determined by quantitating the number of hemolytic foci which appeared in an agar-erythrocyte overlay on thin sections of mouse spleen. After immunization, the number of foci rose to a peak and then declined gradually to levels which persisted through 113 days. At 210 days after immunization, foci activity had merged with control levels. On the other hand, the plaque-forming cell method of Jerne showed an increase in the number of plaque-forming cells until a peak was reached with a subsequent rapid decline to control levels 70-80 days after immunization. It is concluded that the frozen section technique is a sensitive method for the discrimination and enumeration of cells containing specific antibody in tissues.

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Effect of Transplanted Tumor and Various Agents on Liver Regeneration During Pregnancy. (32373)

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In a previous study, the effect of agents which stimulate or inhibit the extent of liver regeneration in partially hepatectomized animals was determined in rats with Walker tumor transplanted directly into the surviving liver at surgery(1). The presence of tumor tended to normalize the regenerative rate in groups treated with several of the agents and except for the depressant action of nicotinamide, tumor growth was little affected in their presence. The current experiments were devised to ascertain the simultaneous effect of pregnancy, a condition under which liver weight restoration is accelerated (2,3) and transplanted tumor on liver regeneration and lesion growth as well as of the combined influence of pregnancy and depressant or stimulating agents on the hepatic process.

Materials and methods. The agents were incorporated into Rockland rat meal on a weight basis, the respective compositions being the same as those employed previously (1): acenaphthene (0.25%; Gesellschaft für

Teerverwertung, Germany), phenylbutazone or Butazoldin® (0.35%; Geigy), thalidomide (0.20%; Merrell), nicotinamide (0.35%) and usnic acid (0.20%); the last two compounds originated from Nutritional Biochemicals Corp.

Holtzman adult female rats were caged with adult males for periods up to 7 days prior to surgery. The gravid and nonpregnant animals under ether anesthesia were partially hepatectomized, two-thirds of the liver being removed(4) and the respective portions dried to constant weight at 100°C. In one series, a standard suspension of Walker tumor cells in saline (volume per rat: 0.050 ml) was injected into the caudal lobe by needle and calibrated syringe according to the detailed procedures reported earlier(1,5); a comparable amount of saline was injected into the caudal lobe of the partially hepatectomized controls. Where spillage occurred at surgery or was definite as noted at necropsy 7 days later, such animals were excluded from consideration. The rats were housed in individual cages and administered diet and water *ad lib.* They were sacrificed (ether) after a period of 7 or 10.5 days and the entire livers removed and dried to constant weight. The gravid animals were at mid to late stages of pregnancy. In rats with tumor, the lesion was completely dissected from the liver and weighed. The amount of tissue regenerated or the increment was calculated by multiplying the dry weight of the liver removed at surgery by the factor 0.46 and subtracting this product from the dry liver weight at necropsy(6). The data from animals suffering body weight losses in excess of about 15% or those of the specified categories in which the tumor did not take or pregnancy

TABLE I. Liver Regeneration in Gravid and Nonpregnant Rats Injected with Walker Tumor and Fed Control and Acenaphthene Diets (Duration: 7 Days).[a,b]

Treatment	No. of rats	Body wt, g ± S.E. At surgery	Body wt, g ± S.E. At necropsy	Liver increment, g ± S.E.	*t*	Wet tumor wt, mg ± S.E.	*t*
Series I-A							
Nonpregnant; without tumor; control diet	23	201 ± 2.5	199 ± 3.6	1.834 ± .065			
Nonpregnant; tumor; control diet	35	198 ± 2.4	202 ± 1.9	1.866 ± .042	0.43	412 ± 46	
Nonpregnant; without tumor; acenaphthene (.25%)	17	201 ± 2.5	199 ± 3.7	2.182 ± .058	5.44**		
Nonpregnant; tumor; acenaphthene (.25%)	32	202 ± 1.8	202 ± 2.2	2.173 ± .049	4.35**	384 ± 29	.51
Gravid; without tumor; control diet	8	224 ± 3.6	232 ± 6.3	2.309 ± .084	3.93**		
Gravid; tumor; control diet	9	217 ± 4.2	236 ± 4.4	2.500 ± .125	5.12** (1.23)[d]	451 ± 10	.35
Gravid; tumor; acenaphthene (.25%)[e]	3	214 ± 2.8	229 ± 7.5	2.463 ± .105	3.38** (.16)[f]		
Nonpregnant; sham-operated; tumor; control diet	31	197 ± 2.6	201 ± 2.8			237 ± 23	
Gravid; sham-operated; tumor; control diet	17	209 ± 4.4	228 ± 8.0			249 ± 53	.05[g]

[a] Except for the last 2 groups which were sham-operated, all animals were partially hepatectomized.
[b] The standard error is given after each ± sign.
[c] Fisher *t* value in the comparison *vs* nonpregnant controls without tumor.
[d] Comparison *vs* pregnant controls with tumor.
[e] Of 18 rats, only 3 were pregnant with tumor weights averaging 445 mg.
[f] Comparison *vs* gravid controls with tumor.
[g] Comparison of the 2 non-hepatectomized groups. For the comparison of both sham-operated nonpregnant as well as gravid rats *vs* the partially hepatectomized gravid controls, the *t* values were 3.51** and 2.07,* respectively. In the same order as stated, the comparison *vs* the nonpregnant operated controls yielded: 3.30** and 2.15.*

** $P < .01$ * $P < .05$

was not evident were excluded.

Results and discussion. Body and wet tumor weights and liver increment data for gravid and nonpregnant animals on the control and 0.25% acenaphthene diets for 7 days appear in Table I. Walker tumor weights are also given for sham-operated gravid and nonpregnant animals in which the cells were transplanted into the caudal lobe at the time of surgery. In agreement with previous findings(1,5), the presence of tumor in the liver did not affect the extent of liver regeneration as compared to the controls without tumor. This was also the case for corresponding groups fed 0.25% acenaphthene diet and for 2 groups of pregnant rats on the control ration. In this experiment, as only one partially hepatectomized rat without tumor and on the acenaphthene diet was pregnant (liver increment: 2.54 g), a comparison could not be made against the corresponding tumor-bearing group. In fact, only 3 rats were available in the latter and as indicated in Table I, the increase in liver increment was in the range of that of the gravid rats with transplanted tumor fed the control diet. The stimulatory action of pregnancy as such on regeneration was more marked than that engendered by acenaphthene feeding. Thus, among the tumor-bearing rats, the increase in increment with the gravid group on the control diet exceeded the value for the nonpregnant rats fed 0.25% acenaphthene (t: 2.87; $p<0.01$).

The growth of tumor transplanted into the livers of the sham-operated group as well as of the respective partially hepatectomized rats was not influenced by pregnancy. However, the tumor weights of the sham-operated animals definitely averaged below those of the subtotally hepatectomized cases. This finding is in line with prior observations in males(5). Also, tumor weights for the rats fed the acenaphthene diet were in the range of the values displayed by the two operated control groups, in corroboration of earlier studies with the above hydrocarbon administered subcutaneously to rats of either sex(1).

Data for gravid and nonpregnant animals fed diets supplemented with usnic acid, phenylbutazone, thalidomide and nicotinamide are presented in Table II. Usnic acid and phenylbutazone rations (Series II-A) caused the usual acceleration in regenerative rate in nonpregnant animals and the increments were even higher in the respective pregnant groups fed the special diets. A comparison with gravid controls was not possible as only two were available. The study was extended to larger groups of animals (Series III-A), the hepatic regenerative stimulants being thalidomide and 2,4-dithiopyrimidine. Similar to the above, as compared to the nonpregnant controls, the liver increments were elevated in the nonpregnant groups on the special diets and even more dramatically in the corresponding gravid animals. However, of the latter, the increase with the groups fed thalidomide was in the range of the gravid controls while the rise in increment with the animals on 2,4-dithiopyrimidine was at the 5% level of statistical significance. As a prior study had shown that in rats undergoing active fetal resorption the extent of liver regeneration was elevated(2), such animals were also considered separately in Series III-A. The findings simulated those of the gravid groups, the synergistic effect with 2,4-dithiopyrimidine persisting ($p<0.02$).

The results with the nicotinamide diet are worthy of comment (Series III-A; Table II). Regeneration was depressed in the nonpregnant animals but as compared to the nongravid controls, the increment was increased in both the pregnant rats and those with fetal resorption. However, these two groups displayed an inhibition of regeneration when compared to the respective controls.

It must be pointed out that this study is beset by several difficulties as emphasized in past reports(1,5). Among others, the partial removal of liver is a rather extreme operation in relation to pregnancy and accordingly, can lead to extensive resorption. Problems are also encountered in tumor cell quantitation and transplantation into the liver coupled with the fact that some of the agents as thalidomide, may provoke definite toxicity and fetal alterations. An evaluation of the latter was not undertaken, the emphasis being directed to the regenerative process as such.

TABLE II. Liver Regeneration in Gravid and Nonpregnant Rats Fed Special Diets (Duration: 10.5 Days).

Treatment	No. of rats	Body wt, g ± S.E. Initial	Body wt, g ± S.E. At necropsy	Liver increment, g ± S.E.	t†	
Series II-A						
Nonpregnant; control	21	211 ± 2.9	233 ± 2.6	1.870 ± .086		
Nonpregnant; usnic acid (.20%)	20	215 ± 2.0	207 ± 4.0	2.584 ± .071	6.26**	
Gravid; usnic acid (.20%)	6	226 ± 2.3	230 ± 7.1	2.958 ± .131		(2.44)*
Nonpregnant; phenylbutazone (.35%)	13	217 ± 1.7	222 ± 3.5	2.412 ± .113	3.90**	
Gravid; phenylbutazone (.35%)	4	227 ± 3.8	245 ± 4.1	2.815 ± .168		(2.63)***
Series III-A						
Nonpregnant; control	27	230 ± 2.2	241 ± 2.0	1.741 ± .067		
Gravid; control	13	240 ± 2.7	302 ± 7.6	2.658 ± .066	8.49**	
Fetal resorption; control	6	240 ± 4.0	256 ±11.0	2.515 ± .216		(.83)
Nonpregnant; thalidomide (.20%)	17	233 ± 2.7	244 ± 3.1	2.027 ± .100	2.47***	
Gravid; thalidomide (.20%)	9	240 ± 3.4	309 ±10.9	2.790 ± .110	7.83**	(1.06)
Fetal resorption; thalidomide (.20%)	10	237 ± 4.0	248 ± 8.1	2.640 ± .227		(.09)
Nonpregnant; nicotinamide (.35%)	13	230 ± 4.4	231 ± 3.1	1.517 ± .038	2.24***	
Gravid; nicotinamide (.35%)	6	237 ± 4.3	265 ±10.1	2.052 ± .114	2.05*	(4.84)**
Fetal resorption; nicotinamide (.35%)	8	243 ± 4.4	248 ± 9.3	2.179 ± .038	3.45**	(5.38)**
Nonpregnant; 2,4-dithiopyrimidine (.050%)	28	230 ± 1.8	241 ± 2.0	2.280 ± .096	4.65**	
Gravid; 2,4-dithiopyrimidine (.050%)	8	239 ± 2.1	300 ± 6.5	2.924 ± .096	8.82**	(2.38)*
Fetal resorption; 2,4-dithiopyrimidine (.050%)	7	242 ± 4.6	268 ± 5.9	2.991 ± .126		(2.62)***

† Comparison against the nonpregnant rats on the control Rockland ration. The t value in parentheses denotes the comparison *vs* the gravid animals fed the control diet.

* $P < .05$ ** $P < .01$ *** $P < .02$

However, under the conditions explored, a unique finding is that pregnancy in partially hepatectomized rats evoked a rather maximalized extent of regeneration and which was little affected by the simultaneous feeding of several known chemical stimulants, except for a synergistic action with 2,4-dithiopyrimidine. The inhibitory effect of dietary nicotinamide on liver regeneration was not completely abolished during gestation.

Summary. Liver regeneration has been investigated in partially hepatectomized nonpregnant and gravid rats with Walker tumor transplanted into the remaining liver at surgery and fed either a control ration or one supplemented with 0.25% acenaphthene over a period of 7 days. Pregnancy accelerated liver weight restoration to a greater extent than acenaphthene feeding and the presence of tumor had no influence on the process. However, the growth of transplanted tumor was more rapid in the partially hepatectomized animals than in the sham-operated gravid and nonpregnant rats. The effect of pregnancy in operated rats on the control diet was maximal, exceeding that produced by various agents known to stimulate liver regeneration and supplemented in diets fed for 10.5 days. These included thalidomide (0.25%), phenylbutazone (0.35%), usnic acid (0.20%) and 2,4-dithiopyrimidine (0.050%). The last diet accelerated the liver weight restoration above that of the control gravid animals but thalidomide contributed

little to the already heightened regeneration when administered to pregnant rats. The depressant, nicotinamide (dietary level: 0.35%), normalized the extent of hepatic regeneration when fed to gravid animals but an inhibitory effect was apparent when the liver increment was compared to that of the pregnant group on the control diet.

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The Submaxillary Secretory Response in the Dog to a Series of Doses of Pilocarpine.* (32374)

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Although pilocarpine has been used widely in the laboratory(1,2,3) and has been given to man(4) to stimulate the secretion of saliva, its dose-response effects on salivary secretion have not been documented. Only the secretory effect of a single dose has been described (5). The present report is based on a study of the dose-response effects of pilocarpine on the dog submaxillary gland.

The administration of a series of increasing doses of pilocarpine was found to result in a flow-rate curve that resembled an inverted-V, indicating that saliva flow rate was suppressed by high doses of pilocarpine. Graham and Stavraky(6), who described a similar response to increasing doses of acetylcholine, showed that high doses of acetylcholine caused glandular vasoconstriction. As it was known that pilocarpine, also, causes vasoconstriction at high doses(7), the drop in response appeared to be due solely to the constrictive effect of the high doses. However, based on the observation that the viscosity of the saliva appeared to increase with dose, the hypothesis was tested that an increase in salivary viscosity at high doses of pilocarpine contributed also to the decline in flow rate.

* This work was done in partial fulfilment of the M. S. degree in pharmacology at Georgetown University.

Materials and methods. Thirty-two dogs of either sex, weighing 3.8 to 11.9 kg, and anesthetized with pentobarbital sodium (30 mg/kg intravenously), were used. A cannula (P.E. 50) was placed in the submaxillary duct at the chorda-lingual triangle(8) and tied in place to avoid loosening and leakage. The trachea and left femoral vein were cannulated. All doses were given intravenously; and each dose was followed by 2 ml 0.9% sodium chloride solution. Dogs 23-32, whose salivary viscosity was determined, were hydrated *via* the femoral cannula throughout the experiment with approximately 50 ml/hr normal saline. A Grass model S4G stimulator was used to stimulate the chorda tympani through a bipolar platinum electrode (Frequency: 20-50 f/s; strength: 5 volts).

The secretory responses of dogs 1-11, 31 and 32 were recorded by counting drops per unit time. The responses of dogs 12-30 were recorded using the sialogram, which is a combined tracing of salivary duct pressure and the rate of flow of saliva. The recording apparatus is demonstrated in Fig. 1.

The recording of flow rate with a transducer was made possible by the attachment of a flexible length of polyethylene cannula (Fig. 1, I) to the outflow end of a pressure transducer. As each falling drop caused the free end of the cannula to bend and rebound,