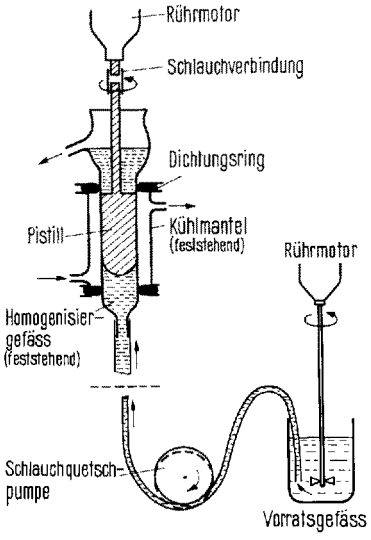
Das Homogenat wird dann in bekannter Weise durch differentielles Zentrifugieren[4] fraktioniert (10 min bei 800 *g*: Kernfraktion als Sediment, 10 min bei 25000 *g*: lysosomenhaltige Mitochondrienfraktion als Sediment, sowie Mikrosomen und Cytoplasma als Überstand). Nach der Kernabtrennung wird die Mitochondrienfraktion durch Zentrifugieren im Durchlaufverfahren (RC-2 Kühlzentrifuge der Fa. Servall mit dem «continuous flow system» nach SZENT-GYÖRGYI und BLUM) gewonnen.



Anordnung für kontinuierliches Homogenisieren. Der Homogenisatorteil ist im Maßstab 1:2,4 abgebildet, die übrige Anordnung im Maßstab 1:10. Die suspendierten Gewebeteilchen werden durch eine Schlauchquetschpumpe (Typ m P2, Bühler, Tübingen) aus dem Vorratsbehälter dem Homogenisator von unten zugeführt. Bei der Homogenisierapparatur nach Potter-Elvehjem der Firma Braun, Melsungen (Rührmotor RM26, von Janke und Kunkel, 60 ml-Homogenisiergefäss mit Teflonpistill) wird das 60 ml-Homogenisiergefäss durch ein für kontinuierliches Homogenisieren modifiziertes ersetzt. Dieses Gefäss aus Pyrexglas trägt zusätzlich Oliven für Zu- und Abfluss des Homogenates und wird von 2 Gummiwülsten getragen, die in eine Metallhalterung eingespannt sind. Die Halterung kann gl ichzeitig als Kühlmantel benützt werden. Die Spaltbreite zwischen Pistill und Glaswand beträgt 0,1 mm. Bei der ersten Passage wird ein konisches Pistill verwendet, das am unteren Teil eine Spaltbreite von 0,2 mm bildet. Das in einem Latexschlauch (⌀ 0,5 cm) geförderte Homogenat wird nur im Bereich der Schlauchquetschpumpe durch 2 Siliconschläuche (⌀ 0,3 cm) geführt.

Die Leistungsfähigkeit des Homogenisators, besonders in bezug auf Schonung labiler Zellorganellen (z. B, Lysosomen), kann durch Bestimmung der sauren Phosphatase[5] in den erhaltenen Fraktionen ermittelt werden, da diese ein charakteristisches Enzym der Lysosomen[5] ist. In der Kernfraktion wurden 14% der Gesamtaktivität gefunden (unzerkleinerte Zellen), im Überstand 7% (zerplatzte Lysosomen?), so dass nach diesem Verfahren etwa 80% der labilen Organellen gewonnen werden können. Die beschriebene Anordnung zum kontinuierlichen schonenden Homogenisieren ist allgemein für Gewebe geeignet, die eine ähnliche Konsistenz wie Nierengewebe haben und schafft die Möglichkeit, grössere Mengen von Zellorganellen zu gewinnen, um deren Komponenten näher zu untersuchen.

Summary. Large-scale preparations of tissue homogenates are not conveniently obtained with the conventional Potter-Elvehjem homogenizer. Therefore this homogenizer was modified to permit a continuous pump-driven passage of material through the homogenizer without undue damage to particulate fractions of cells.

E. MEHL

Biochemische Abteilung der Deutschen Forschungsanstalt für Psychiatrie, Max-Planck-Institut, München (Deutschland), 9. Juni 1965.

[4] W. C. SCHNEIDER, J. biol. Chem. *176*, 259 (1948).
[5] H. BEAUFAY, P. JACQUES, P. BAUDHUIN, O. Z. SELLINGER, J. BERTHET und C. DE DUVE, Biochem. J. *92*, 184 (1964).

STUDIORUM PROGRESSUS

Thalidomide Malformations and Genetic Background in the Rabbit

Introduction. The value of thalidomide as a tranquilizer and its identification as a teratogenic agent responsible for a widely publicized increase in external and internal malformations in human fetuses have posed challenging problems to both the chemist and the biologist. To the chemist, who is aware of the close relationship between thalidomide and numerous substances of everyday human use which normally are harmless, the *major question* concerns the chain of reactions by which it or its metabolites can become teratogenic. To the biologist, one perplexing question concerns the seemingly limited vulnerability to this drug displayed by those mammalian species most often used in testing teratogens, whereas the rabbit, which has rarely been used in such studies, appears to compare favorably with the human in that respect. This report concerns (a) a preliminary study of the rabbit's response to thalidomide and (b) a test for genetic differences in vulnerability.

Materials and methods. Three strains (III, New Zealand White, 3.9 kg; M, mixed New Zealand White breeding, 3.6 kg; and OS, Dutch origin, 3.3 kg) maintained at The Jackson Laboratory are involved. A total of 25 control and 18 experimental litters were produced. The reported incidence of spontaneous deformity of III and OS is 4 and 5% respectively[1].

Thalidomide was administered orally in gelatin capsules at the rate of 500 mg/day/doe on days 6–11 post coitus (pc) (one litter was treated days 4–11). Capsules were so placed by a balling gun that swallowing was immediate with negligible trauma; hence placeboes were not

[1] P. SAWIN and D. CRARY, Clin. Orthopaedics *33*, 71 (1964).

given to controls. Treated (T) and control (C) animals were otherwise treated alike. All were weighed three times and palpated for pregnancy at 10–14 days pc. Parturition was induced by hormone (Armour's posterior pituitary extract) administration on day 31 pc in all but two cases where the does were sacrificed. External examination was made immediately, followed by internal examination of the soft tissues and subsequently by alizarin staining of the skeleton[2]. Both control and treated litters were obtained from 10 of the mothers. In 14 cases the control gestation followed a previously treated mating; in 11 cases it preceded it.

Results. Reproduction was reduced in all treated groups and varied with strain (Table I). In race III a slight reduction in litter size and a significant reduction in percentage of live young were observed. The high proportions of dead, stillborn, and particularly resorbed young indicates a broad spectrum of abnormality with varying degrees of compatibility with life. The OS had small litters but the highest proportion of live young, both in treated and control groups. Effects upon reproduction apparently were much less in the treated M groups, but there was a high proportion of stillborn young. The high parturitional mortality in the controls of this group could not be due to thalidomide.

There was a much higher incidence of spontaneous malformation among the controls (Table II) than other investigators report[3–8]. This may be due to more extensive examination, but is also due in part to the racial background. Abnormalities of sutural bones, sternebrae, and tail are normal variations[1,9]. In analysis, their omission increases the proportion of normal offspring in both treated and control groups (see weighted data, Tables I and III). The increase in treated groups is minimal except in the M group where nearly 40% are normal by weighting. Such decrease in abnormality may be attributed to hybrid vigor. Treated and control populations differ significantly ($P < 0.01$) in both weighted and unweighted III and M groups, but only in the weighted OS group (Table III).

Strain differences in vulnerability to malformation. Malormations, both spontaneous and induced, are grouped under seven categories (Table II). Some external variat'ons, for example short and kinky tails in category 1, are observed as asymmetries and abnormal fusions of centra and hemi-centra in category 2. Similarly repetition occurs in categories 3 and 4.

(1) External body malformations: The syndrome of anomalies induced externally in these three genotypes appears to have a certain degree of regional specificity. These malformations are localized at opposite extremes of the animal, i.e. head vs tail and dorsal vs ventral. Specifically, race III shows ear deficiency, whereas OS shows deficiencies of the eyes and nose. The M group tends to show schisis both dorsally (Figure 4) and ventrally, and otocephaly. All groups show tail malformations in controls as well as treated, indicating that in these cases thalidomide may be aggravating an existing vulnerability.

(2) Internal axial skeleton: Malformations, some of which are strain specific, are localized in head and tail and ventrally in the sternum. Treated young of races III and OS have a high proportion of sutural bones in the skull and of malformation of tail vertebrae. These are also found to some extent in the controls. Sternebral variations likewise occur in both treated and control populations, but whereas skull and tail abnormalities increase with treatment, the sternal variations decrease in incidence (III, 27% in C, 21% in T; OS, 72% C, 47% T; M, 23% C, 15% T). Irregular notches in the neural arches of the axis in M and OS and fusions in neural arches of III are minor additional variations of treated animals not found in controls. The high incidence of sutural bones in treated animals was anterior between nasals or frontals, whereas in the race III controls it was posterior between the parietals. This, together with a tendency to displace the anterior fonticulus, indicates a shifting effect of the

[2] D. Crary, Stain Technol. *37*, 124 (1962).
[3] G. Somers, Lancet *1962 i*, 912.
[4] G. Somers, Orvosi Hetilap *103*, 2456 (1962).
[5] D. Felisati, Lancet *1962 ii*, 724.
[6] E. Thissen, Münch. med. Wschr. *47*, 2282 (1962).
[7] T. Ingalls, F. Curley, and P. Zappasodi, New England J. Med. *271*, 441 (1964).
[8] A. Dekker and A. Mehrizi, Bull. Johns Hopkins Hosp. *115*, 223 (1964).
[9] E. Peck and P. Sawin, J. exp. Zool. *114*, 335 (1950).

Table I. Effects on reproduction

Number	III				OS				M				Totals			
	T		C		T		C		T		C		T		C	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Mothers	11		14		12		9		7		4		30		27	
Litters	8	(50)	18	(69)	5	(29)	4	(31)	5	(45)	3	(43)	18	(41)	25	(54)
Mating failures	7	(44)	8	(31)	10	(59)	9	(69)	6	(55)	4	(57)	23	(52)	21	(46)
Total litter resorbed	1	(6)	0		2	(12)	0		0		0		3	(7)	0	
Viable young	24	(44)	121	(86)	16	(62)	16	(89)	37	(77)	14	(41)	77	(60)	151	(79)
Dead	9	(16)	12	(9)	0		2	(11)	0		11	(32)	9	(7)	25	(13)
Stillborn	10	(18)	6	(4)	3	(12)	0		10	(21)	4	(12)	23	(18)	10	(5)
Total classifiable young	43		139		19		18		47		29		109		186	
Resorbed in viable litters (unclass.)	12	(22)	1	(1)	7	(27)	0		1	(2)	5	(15)	20	(16)	6	(3)
Total young	55		140		26		18		48		34		123		192	
Litter size (total pop.)	6.7		7.8		5.2		4.5		9.6		11.3		7.1		7.7	
Litter size (♀♀ T & C)[a]	7.1		8.1		5.2		4.7		11.0		12.5		7.1		8.0	
Normals	1	(3)	85	(61)	0		2	(11)	3	(6)	21	(72)	4	(4)	108	(58)
Weighted normals	2	(5)	138	(99)	2	(11)	18	(100)	18	(38)	29	(100)	22	(21)	185	(99)

[a] Includes only those females that had both treated and control litters.

Table II. Numerical incidence of malformations listed by strain and treatment

Abnormalities	III		OS		M		Total	
	T	C	T	C	T	C	T	C
(1) External body malformations								
Otocephaly					1		1	
Ears small or notched	2				4		6	
Coloboma of the iris			2				2	
Microphthalmia			3				3	
Nares – closed			1				1	
Spina bifida					1		1	
Thoracoschisis					1		1	
Lateral hernia	1						1	
Tail – kinky	5		5		19	2	29	2
Tail – short	9		14	8	3		26	8
(2) Internal axial skeleton								
Nasal bones asymmetrical	1						1	
Frontals bossed	2						2	
Suture bones								
nasofrontal	24	3	10	5	8		42	8
parietal	3	7			3		6	7
Atlas – anterior arch								
bipartite	1						1	
Axis neural arch notched			4		6		10	
Ventral spinous processes asym.	1						1	
Sternebrae								
reduced or absent	2	29	1	6		5	3	40
bipartite – bilaterally	1	8		3	2	2	3	13
bipartite – dorsoventr.	6						6	
fused			8	4			8	4
asymmetrical	1				5		6	
Xiphisternum abnormal					1		1	
Ribs								
irregular	3		2		1		6	
on v. 7					1		1	
poor cartilage connection	1						1	
Tail centra								
asymmetrical	8	2	4		12		24	2
hemi		6	3	1	4		7	7
bipartite					6		6	
fused	20	5	2		11		33	5
Tail neural arches								
asymmetrical	1						1	
fused	1						1	
(3) External limbs								
Forefeet – clubbed	3		4		10		17	
Thumbs								
reduced or absent	12		7		11		30	
at right angles	10		1		8		19	
fingerlike	3						3	
Fifth finger – absent	1						1	
Hind feet – clubbed	5		3		8		16	
Phocomelia					1		1	
(4) Internal limbs								
Scapular								
spine – crumpled					1		1	
Radius – short					2		2	
1st metacarpal – absent or reduced	9		5		7		21	
1st prox. phalanx – reduced or absent	7		1		3		11	
1st medial phalanx present	3						3	
Phalanges bipartite					1		1	
Tibia – reduced or absent			1		4		5	
2nd Metatarsal – reduced					1		1	
(5) Internal G.I.								
Stomach								
displaced	11						11	
small	5						5	
Left post. lobe liver absent	1						1	
Gall bladder double					3		3	
Colon								
with diverticula	7						7	
sacculate	15						15	
ruptured			1				1	
Cloaca					1		1	
(6) Internal U.G.								
Kidney								
reduced or absent	11				3		14	
at right angles	1						1	
Ureter – behind vena cava	2						2	
Bladder – small	1						1	
(7) Vascular and pulmonary								
Aortic arch								
broad	3		1				4	
fused with pulmonary arch			2				2	
turns right then left			1				1	
Pulmonary artery								
behind aorta			1				1	
absent		1						1
Heart								
small	4						4	
auricle – small	1						1	
Lungs – small	3						3	
Jugular – absent	1						1	
Vena cava								
displaced	1						1	
double			1				1	
Renal vein – double			1				1	

Numerals in columns refer to the total number of classifiable young exhibiting a specific abnormality. It should be noted that a single animal may have more than one anomaly and therefore will occur more than once in the Table.

Table III. Chi square test of the proportion of abnormal animals

Test group	Unweighted Col. I			Weighted Col. II		
	χ^2	d.f.	P	χ^2	d.f.	P
III (T vs C)	40.05	1	< 0.01	157.54	1	< 0.01
OS (T vs C)	2.11	1	> 0.05 (N.S.)	30.01	1	< 0.01
M (T vs C)	35.90	1	< 0.01	29.10	1	< 0.01
Treated (III vs OS vs M)	1.69	2	> 0.05 (N.S.)	15.10	2	< 0.01
Control (III vs OS vs M)	19.56	2	< 0.01	0.431	2	> 0.05 (N.S.)

variations under drug influence, even though there is no discernable change in incidence of sutural bones of treated vs controls in the parietal region.

(3) and (4) External and internal malformations of the limbs: In these two groups no comparable variation is found in the controls. The abnormalities are reduction or displacement of distal parts (Figure 1) of either a varus or hemimelic type (Figure 4) in both fore and hind limbs.

Accessory ossification centers appear twice, one of which is biparted laterally.

(5) and (6) Malformations of the G.I. and U.G. tracts: Malformations were found only in treated animals, and of the groups race III is significantly the more vulnerable (Figures 2, 3, 6, 7, 10). Abnormalities were of four types: (a) displacement of organs (stomach, Figures 3, 6, 7): (b) miniaturization or complete absence of organs (stomach, Figures 3, 6, 8; kidney, Figures 6, 8); (c) duplication or additional structures (double gallbladder, sacculi and diverticula of colon, Figure 10); and (d) failure of an early developmental stage (persistent cloaca, Figure 10).

(7) Vascular and pulmonary malformations: Race III and OS are both seriously affected in this region; III by miniaturization of heart and lungs (Figures 2, 6–8) and OS by changes in vascular type (Figures 2, 9). The one control anomaly (pulmonary artery absent) suggests a localized inherent weakness in race III accounting for, in part at least, the high incidence of abnormalities in this area in the treated animals.

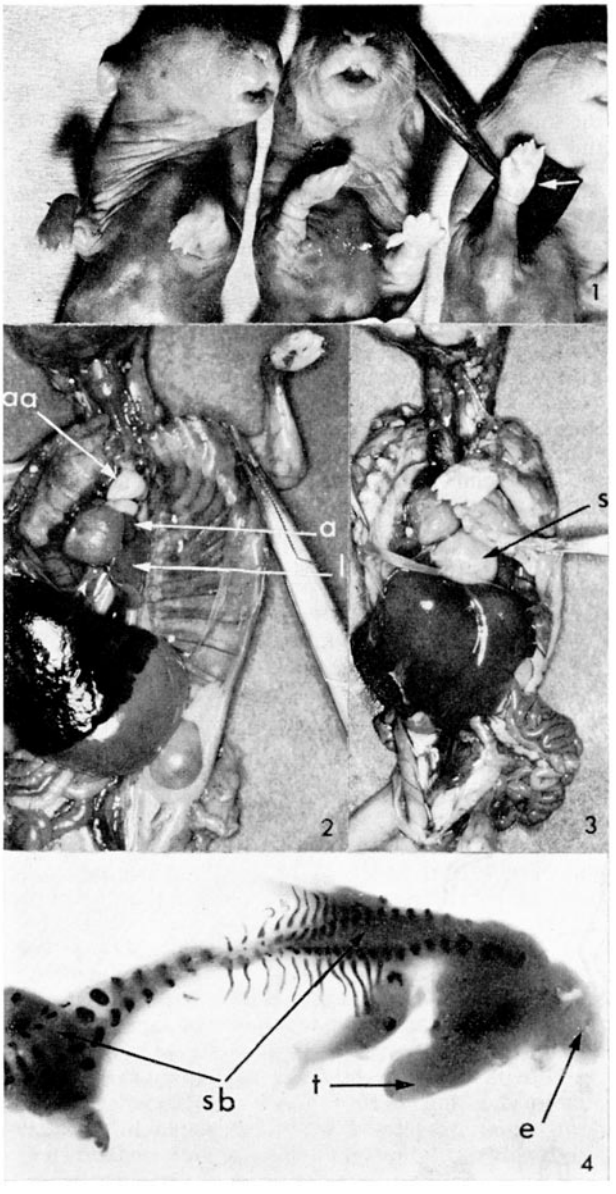


Fig. 1. Note bulbous thumbs of first two rabbits which project at right angles from hand. Note also vestigial thumb (rabbit at right) (T1, 2, and 3, M group).

Fig. 2. Note small size of auricles (a) and lungs (l), also broad aortic arch (aa), and absence of left jugular vein (T175, race III).

Fig. 3. Note small stomach (s) in thoracic cavity just below heart and separated from the liver by the diaphragm (T182, race III).

Fig. 4. Phocomelic. Dorsal view. Note also otocephaly with encephalocele (e), large protruding tongue (t), wavy ribs, and spina bifida (sb) (T70, M).

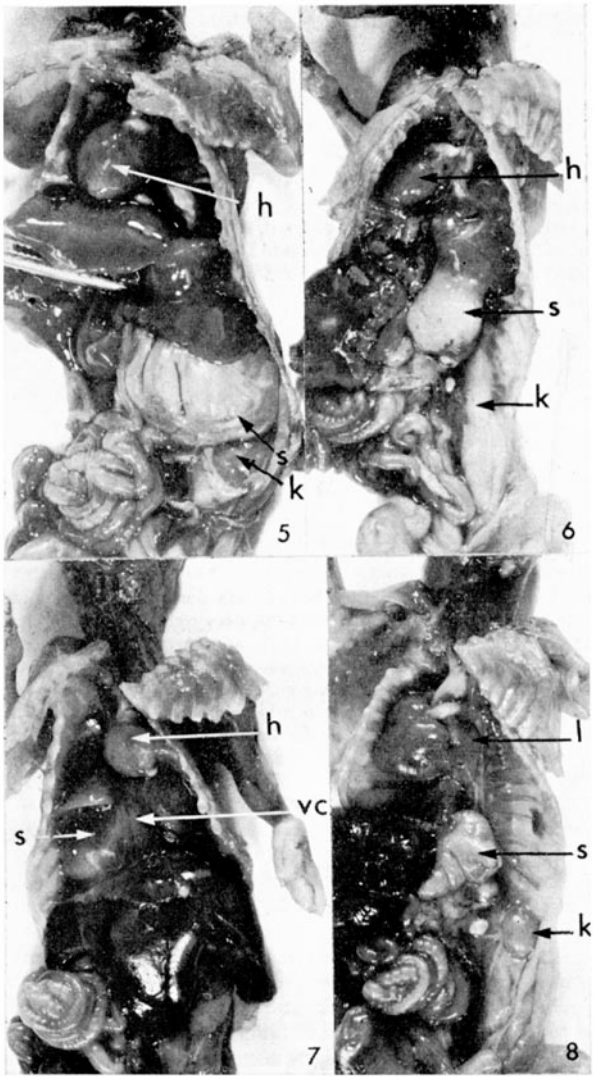


Fig. 5. Normal control (865X, race III). Note relative size of heart (h), stomach (s), and kidney (k), and compare with Figs. 2, 3, 6–7.

Fig. 6. Note absence of left kidney (k), small heart (h), and small stomach (s) partially in thoracic cavity (T91, race III).

Fig. 7. Note small heart (h), large stomach (s) in thoracic cavity and behind anterior vena cava (vc) (T93, race III).

Fig. 8. Note relatively large heart, but small lungs (l), stomach (s), and kidney (k) (T94, race III).

Symbols

Symbols: a = auricle, l = lung, aa = aortic arch, s = stomach, k = kidney, h = heart, vc = vena cava, t = tongue, e = encephalocele, sb = spina bifida.

The significance of the differences between treated and control groups is shown in Table III. In the weighted data, all animals having malformations only in dorsal skull, sternum, or tail are considered normal since these regions are vulnerable to spontaneous abnormality. There are no significant differences between treated and control populations in the OS race and no strain difference in unweighted treated groups. However, when allowance is made for the spontaneous strain specific vulnerability, there is left a definite and significant racial effect in response to thalidomide, due possibly to increased genetic variability in the M group. There are, however, also obvious differences in types of anomalies (Table II).

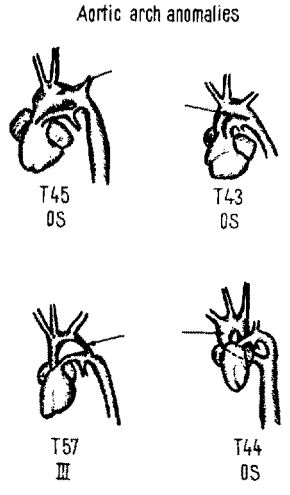


Fig. 9. Aortic arch anomalies. Note broad aortic arch of T45, aortic arch fused to pulmonary artery (T43), very reduced aortic arch and persistence of ductus arteriosus (T57), and aortic arch of T44 which turns right instead of left and then loops back to the left side under the heart, joining the descending aorta below the pulmonary artery. In this case, the left subclavian arises from the pulmonary artery instead of the aortic arch.

Discussion. Our observations confirm previous reports[3-6,10-12] which indicate that the limbs are generally the most affected part. The higher incidence and greater range of malformation in our study, including racial differences in kind, localization, and interrelation with growth processes, are of special significance. In several cases investigators gave no evidence that internal organs had been examined. However, we found a substantial number of internal variations, even in our control litters, involving tail vertebrae, sutural bones, and sternebrae (of minor clinical importance). This suggests that without a thorough examination many thalidomide effects of important etiological or clinical significance may be overlooked. This conclusion is supported by a recent report meticulously describing the effects of thalidomide in a 13-month-old child[13]. A broad spectrum of highly localized malformations is shown involving bone, cartilage, and associated muscles, nerves, and blood vessels, and displacement of the kidneys. Most serious, from the clinical viewpoint, is the finding of thalidomide defects in the vital internal organs. Some of these are undoubtedly lethal, and undiscovered miniaturization or displacement of organs could be a source of severe physical handicapping in later life. The tendency for (a) a shift in the racial positions of the anterior fonticulus and sutural bones of the skull, (b) for decreased incidence of sternal anomalies, and (c) for increased incidence of tail anomalies as thalidomide effects are important in revealing the mode of action of the drug by way of the species, strain, or individual differences in growth processes. It is of significance with reference to time of drug administration that anomalies tend to fall at either end of the primary anteroposterior gradient or at the distal ends of the transverse gradients. That time is a factor in determining malformation is well established in man, and also has been shown for the rabbit[7,8]. The

[10] K. SPENCER, Lancet *1962 ii*, 100.
[11] M. SELLERS, Lancet *1962 ii*, 249.
[12] A. GIROUD, H. TUCHMANN-DUPLESSIS, and L. MERCIER-PAROT, C. r. Soc. Biol. *156*, 765 (1962).
[13] E. HAGEN, Can. med. Assoc. J. *92*, 283 (1965).

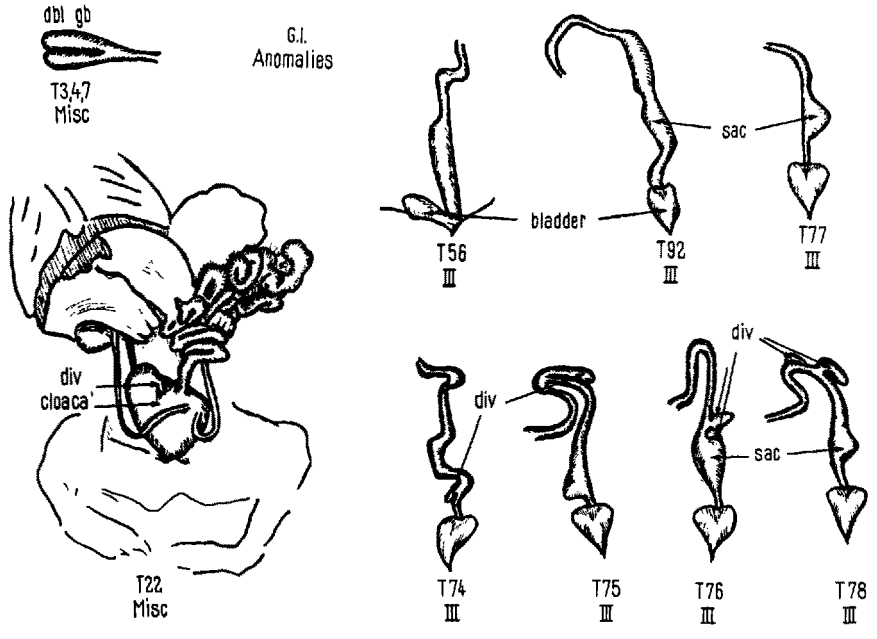


Fig. 10. Anomalies of the gastrointestinal tract. Note in upper row the double gallbladder and variations of sacculate colons. Lower row shows diverticula as well as sacculi of the colon and at the left a persistent cloaca, a result of the failure of the formation of the urorectal septum.

critical period in the rabbit during which administration of thalidomide is effective is not yet defined. It must be, however, very close to the times at which implantation, metamerism, and differentiation of the heart and adjacent vessels are nearing completion (8–9 days pc). This could account for the interesting syndrome of effects. It would also account for the interracial differences in syndrome and in number of defects, since there are fewer soft tissue anomalies in the OS and M strains than in III. This indicates the importance of knowledge of relative residual vulnerability to spontaneous malformation with reference to time and localization in strains used in drug testing. With one exception all of these animals were treated at the same embryonic age. The observed strain differences may indicate differential maturation of organs and tissues as the critical element determining localized vulnerability. Tabulation and analysis of control litters shows that treatment previous to current pregnancy has no significant residual effect on succeeding litters in all except the sternebral variation (reduced or absent 5th or 6th sternebrae) in strain III. A certain proportion of the abnormalities found in control populations, with one exception, fall in areas known from previous growth studies[9] to be vulnerable to retardation and thus have genetic origin. When the treated data are weighted for strain specific differences in vulnerability which cannot be due to thalidomide there remain highly significant strain differences in response to the drug[14].

Zusammenfassung. Thalidomid wurde in Gelatinkapseln peroral (500 mg/Tag/Kaninchen) an 30 gravide Kaninchen vom 6. bis 11. Tag nach Konzeption verabreicht. Von 109 klassifizierbaren Jungen waren nur 4% normal: 77 überlebten, 9 waren tot und 23 wurden abortiert; 20 weitere waren teilweise resorbiert und deshalb unklassifizierbar. Die drei genetisch verschiedenen Kaninchenstämme des Versuchs wiesen signifikante Unterschiede auf in Typus, relativer Zahl und Verteilung der Missbildungen.

P. B. Sawin, D. Crary,
R. R. Fox, and H. M. Wuest

Jackson Laboratory, Bar Harbor (Maine) and Sloan-Kettering Institute for Cancer Research, New York City (USA), June 17, 1965.

[14] This investigation was supported in part by Public Health Service research grant CA-00281 from the National Cancer Institute and by an allocation from Public Health Service General Research Support Grant No. 1 SO1 FR-05545-01 to the Jackson Laboratory, Bar Harbor (Maine).

Antibody Production by Blood Leucocytes

Introduction. Little is still known of the internal mechanisms of the cell which result in antibody formation, but there is considerable evidence for the presence of antibody-forming cells within the architecture of lymphoid organs, such as spleen and lymph nodes (Gowans and McGregor[1], Humphrey and White[2]). Production of antibody can also occur locally in almost any tissue provided antigen is present. These facts have been established by ablation of organs, by irradiation, by damage of lymphoid organs and by the extraction of antibody from tissues; much additional information was also obtained by transplantation of tissues from immunized animals to normal inbred or irradiated recipients.

It is the purpose of this communication to consider recent evidence for antibody-producing cells in yet another location, namely in lymph and blood. Gowans and McGregor[1] have summarized the data which leave no doubt that antibody-producing cells can be found in the circulation. Some of the recent studies in our laboratories (Hulliger and Sorkin[3,4], Landy et al.[5]) attest by quite separate means to the antibody-producing capacity of peripheral blood leucocytes. Some implications of these findings and the possibilities they raise for future work will be considered.

Methods. The experimental situations we investigated were (a) rabbits hyperimmunized with human serum injected intravenously, blood leucocytes being obtained for test 4 days after a booster injection, and (b) rabbits given a single intravenous injection of 5 μg of *Salmonella enteritidis* somatic polysaccharide, blood leucocytes being taken for test at daily intervals thereafter. In (a) de novo synthesis of antibody in vitro was demonstrated by incubation of 40–80 · 10^6 nucleated blood leucocytes with C^{14}-amino acids for 3 h and the incorporation into specific antibody was measured by the radioactivity present in the antigen-antibody precipitate produced by carrier human serum albumin-rabbit anti-HSA. In (b) the production of specific antibody was shown by a modification of the technique of localized hemolysis in gel (Jerne et al.[6]) in which 5–20 · 10^6 washed blood leucocytes in Eagle's medium plus agar were mixed with sheep erythrocytes that had been coated with *S. enteritidis* polysaccharide, the mixture poured into petri plates and allowed to solidify, incubated, and the plates overlaid with guinea-pig complement. The resultant zones of hemolysis (plaques) around individual leucocytes were specific for *S. enteritidis* polysaccharide in that plating the same leucocytes on normal sheep red cells, or on red cells coated with an immunologically unrelated somatic polysaccharide yielded no plaque formation.

Results. Twenty hyperimmunized rabbits were examined for antibody production by splenic and peripheral blood cells and appreciable antibody synthesis by blood leucocytes was demonstrated in 12 of the animals (Hulliger and Sorkin[3,4]). Some illustrative data are given in Figure 1.

[1] J. L. Gowans and D. D. McGregor, Progr. Allergy *9*, 1 (1965).
[2] J. H. Humphrey and R. G. White, in *Immunology for Students of Medicine* (Blackwell Scientific Publications, Oxford 1963), p. 135.
[3] L. Hulliger and E. Sorkin, Nature *198*, 299 (1963).
[4] L. Hulliger and E. Sorkin, Immunology *9*, in press (1965).
[5] M. Landy, R. P. Sanderson, M. T. Bernstein, and A. L. Jackson, Nature *204*, 1320 (1964).
[6] N. K. Jerne, A. A. Nordin, and C. Henry, in *Cell-Bound Antibodies* (Eds., B. Amos and H. Koprowski; The Wistar Institute Press, Philadelphia 1963), p. 109.