Mixed couples composed of individuals from the thelygenous strain R (generation 10) and by individuals from the arrhenogenous strain K (generation 9) produced, on the other hand, an average number of 4.41 eggs per day. The total amount of fertilized eggs was 4543.

Fertility has thus nearly doubled (Figure) in the mixed couples.

Such markedly different results must be attributed to the above-mentioned sexual interactions. They re-establish in the mixed couples that balance which had been altered by selection within the arrhenogenous and thelygenous lines and cannot be established in couples composed of individuals that have exceedingly similar sex genotypes.

The interpretation advanced previously that a certain dose of genes for one sex is necessary for the normal expression of the genes of the other sex is thus demonstrated in *Ophryotrocha*, and the occurrence of genetic sex bipotency in all kinds of eusexual organisms can be interpreted on a functional basis.

A system of control and feedback mechanism through sex hormones among individuals of both sexes and controlled through different regulating systems has been accordingly proposed[6,7].

Riassunto. Si dimostra che interazioni fra individui che posseggono genotipi sessuali sbilanciati in seguito alla selezione per geni sessuali maschili e femminili portano ad un subitaneo aumento della fertilità a causa del ristabilirsi dell'equilibrio genetico. Ciò dimostra che in *Ophryotrocha* una certa dose di geni di un sesso è necessaria per la normale attivazione dei geni dell'altro sesso e fornisce una interpretazione funzionale al fenomeno della bipotenzialità genetica dei sessi.

G. Bacci and O. Bortesi

Istituto di Zoologia, Università di Torino (Italy), 27th January 1967.

[6] G. Bacci, Riv. Biol., in press.

[7] The work has been supported by a grant from the Italian National Research Council.

Differences in Teratogenic Response and in Capacity to Repair in Embryos of Two Inbred Strains of Mice

Studies comparing the susceptibilities of different inbred strains of mice to teratogenic agents have disclosed that in the experimental production of congenital abnormalities, the frequency as well as the pattern of malformations is determined in part by the genetic background of the embryo. Reference is made to the work of Callas[1], Dagg[2,3], Fraser[4,5], and Fraser and Fainstat[6].

The present investigation addressed itself to the question whether the variability in response to a teratogenic agent by inbred strains of mice is related to inherent strain differences in developmental rates, or whether the variability in teratogenic response is a reflection of differences in capacity for repair during embryogenesis.

[1] G. Callas, Anat. Rec. *142*, 336 (1962).
[2] C. P. Dagg, Am. J. Anat. *106*, 89 (1960).
[3] C. P. Dagg, Am. Zoologist *3*, 223 (1963).
[4] F. C. Fraser, 1st Int. Conference on Malformations (J. B. Lippincott Co., New York 1961), p. 179.
[5] F. C. Fraser, 2nd Int. Conference on Malformations (Int. Med. Congress, Ltd., New York 1964), p. 277.
[6] F. C. Fraser and T. D. Fainstat, Pediatrics, Springfield *8*, 527 (1951).

Table I. Incidence and pattern of trypan blue induced malformations in C57Bl/6J mice

Group	Age of fetuses[a] at treatment	Age of fetuses[a] on day sacrificed	No. of fetuses recovered	Mean litter size	Mean crown-rump length mm	No. of normal fetuses	No. of abnormal fetuses	% of abnormal fetuses
Controls								
1	7	7	16	8.5 ± 0.35	0.8 ± 0.05	16	0	0.0
2	7	8	26	6.5 ± 0.25	1.6 ± 0.20	26	0	0.0
3	7	9	23	7.7 ± 1.36	2.6 ± 0.24	23	0	0.0
4	7	10	20	5.0 ± 0.79	4.8 ± 0.10	19	1	5.0
5	7	11	19	6.3 ± 1.78	6.7 ± 0.09	19	0	0.0
6	7	12	25	6.3 ± 1.08	7.6 ± 0.23	21	4	16.0
Experimentals								
7	7	10	15	7.5 ± 1.64	2.3 ± 0.01	0	15	100.0
8	7	11	18	5.7 ± 0.54	2.4 ± 0.16	0	18	100.0
9	7	12	18	6.0 ± 0.93	2.3 ± 0.13	0	18	100.0

In breaking down abnormalities into the various categories many cases were listed more than once, whenever more than one type of malformation occurred in the same embryo. This fact should be borne in mind when adding the figures of Table I. [a] The age of the fetus is calculated by counting the day the copulation plug is noted as day 1.

Table II. Incidence and pattern of trypan blue induced malformations in Swiss albino mice

Group	Age of fetuses[a] at treatment	Age of fetuses[a] on day recovered	No. of fetuses recovered	Mean litter size	Mean Crown-rump length mm	No. of normal fetuses	No. of abnormal fetuses	% of abnormal fetuses	Type of abnormality Head %	Tail %	Misc. %
Controls											
10	7	7	31	10.3 ± 0.26	1.4 ± 0.08	31	0	0.0	0.0	0.0	0.0
11	7	8	27	9.3 ± 0.61	2.5 ± 0.10	27	0	0.0	0.0	0.0	0.0
12	7	9	40	10.0 ± 1.06	3.2 ± 0.23	40	0	0.0	0.0	0.0	0.0
13	7	10	75	12.5 ± 0.91	4.6 ± 0.10	74	1	1.3	0.0	1.3	0.0
14	7	11	37	9.3 ± 1.29	6.4 ± 0.12	36	1	2.7	2.7	0.0	0.0
15	7	12	48	9.6 ± 1.28	8.9 ± 0.27	46	2	4.2	0.0	2.1	6.3
16	7	Birth	66	7.8 ± 0.64	–	66	0	0.0	0.0	0.0	0.0
Experimentals											
17	6	10	46	11.0 ± 0.77	5.5 ± 0.10	15	31	67.4	37.0	30.4	13.0
18	6	11	38	12.7 ± 0.97	7.2 ± 0.20	25	13	34.2	13.2	18.4	18.4
19	6	12	33	8.3 ± 1.43	9.1 ± 0.16	20	13	39.4	24.2	3.0	30.3
20	7	10	77	9.6 ± 0.49	5.3 ± 0.13	36	41	53.2	15.6	48.1	10.4
21	7	11	133	10.2 ± 0.98	7.4 ± 0.07	86	47	35.3	18.0	16.5	10.5
22	7	12	30	10.0 ± 2.35	9.8 ± 0.08	20	10	33.3	16.7	6.7	20.0
23	8	10	98	10.9 ± 1.17	5.2 ± 0.56	30	68	69.4	10.2	56.1	16.3
24	8	11	39	11.0 ± 0.47	6.6 ± 1.12	17	22	56.4	38.5	23.1	17.9
25	8	Birth	43	5.6 ± 0.19	–	33	10	23.3	0.0	23.3	0.0

In breaking down abnormalities into the various categories many cases were listed more than once, whenever more than one type of malformation occurred in the same embryo. This fact should be borne in mind when adding the figures of Table II. [a] The age of the fetus is calculated by counting the day the copulation plug is noted as day 1.

Materials and Methods. C57B1/6J and Swiss albino strains were chosen for comparison. The dye trypan blue was used to induce malformations.

Female mice of each strain were injected i.p. on the 7th day of pregnancy with 0.4 ml of 1% solution[7] of trypan blue[8]. Controls received 0.4 ml of 0.9% solution of physiological saline. Embryos were removed on the 9th, 10th, 11th and 12th days of gestation.

All embryos were examined grossly for malformations under a Spencer dissecting scope, and the crown-rump length was measured in mm with a laboratory ruler.

Results. Teratogenesis in C57B1/6J mice. All 51 embryos recovered from C57B1/6J females injected with trypan blue were malformed (Table I, group 7–9) and showed complete developmental arrest, but were alive, as indicated by the presence of a rhythmically beating heart. The mean crown-rump measurements in control embryos of the C57 strain increased from 2.6 mm at 9 days to 7.6 mm at 12 days of fetal age (Table I, group 3 and 6; Table III, group 3 and 6), while experimental embryos, as late as the 12th day never exceeded a mean crown-rump length of 2.3–2.4 mm (Table I, group 9; Table III, group 6 and 9).

Teratogenesis in Swiss albino mice. 260 embryos out of a total of 553 embryos showed malformations of the anterior and posterior axis. There was no significant difference in frequency nor in the types of malformations produced when treatment was initiated on either days 6, 7, or 8.

The frequency of different types of malformations recovered changed with advancing fetal age. There were fewer malformations recovered on the 12th day of gestation than on the 10th day (Table II, group 17 and 19, 20 and 22, 23 and 25), while the litter size remained relatively constant during this period.

Comparison of developmental rate of control Swiss albino and C57B1/6J mice. A comparison of the 2 strains

Table III. *t* Test for significance between 2 means (crown-rump length)

Groups	*p*	
3 and 6	0.001	Significant
6 and 9	0.001	Significant
1 and 10	0.001	Significant
2 and 11	0.001	Significant
3 and 12	0.100	Not significant
4 and 13	0.200	Not significant
5 and 14	0.250	Not significant
6 and 15	0.001	Significant

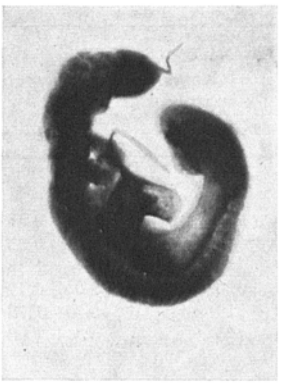

Fig. 1

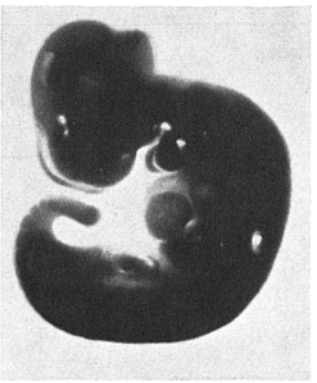

Fig. 2

[7] This dosage was selected because preliminary experiments revealed it to be sublethal but highly teratogenic for the C57B1/6J and Swiss albino strains.

[8] The trypan blue was obtained from Matheson Coleman and Bell Company.

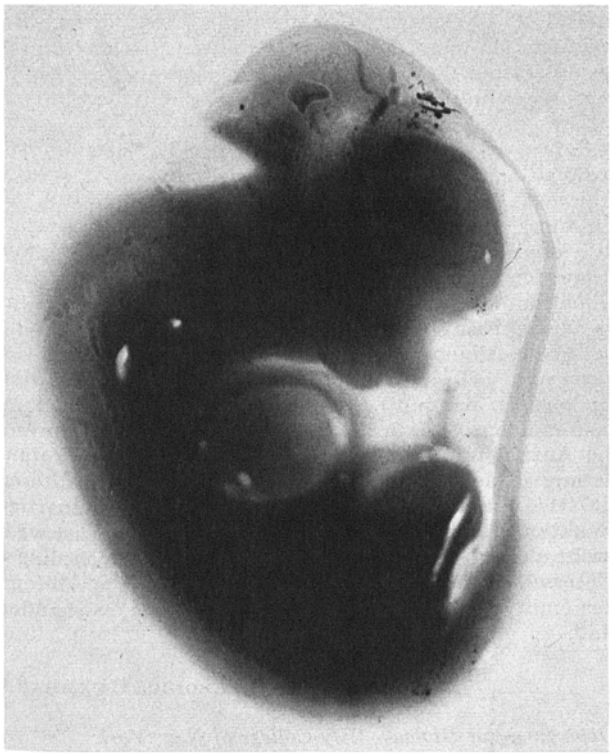

Fig 3

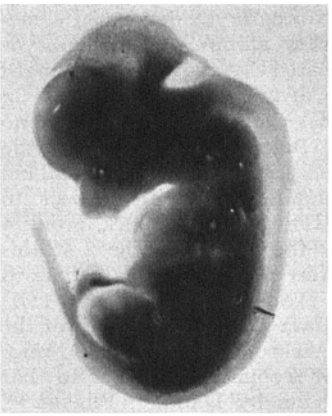

Fig. 8

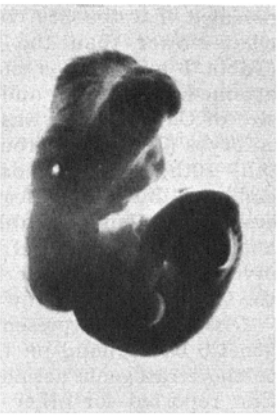

Fig. 9

Fig. 10

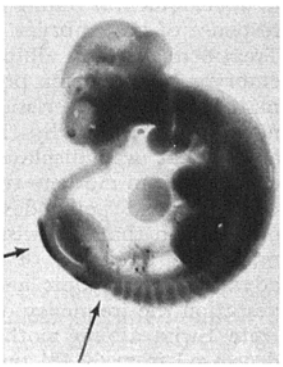

Fig. 11

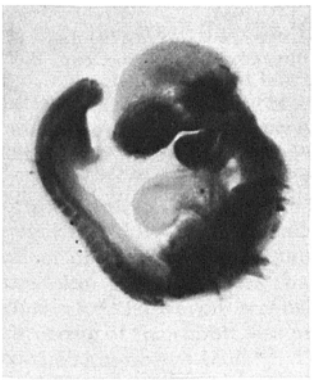

Fig. 4

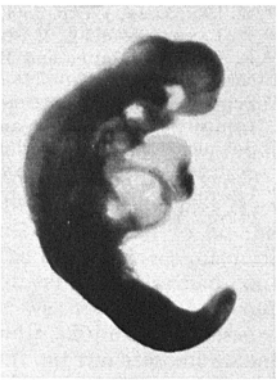

Fig. 5

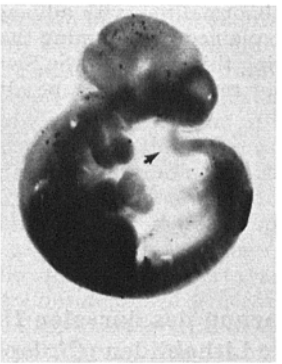

Fig. 12

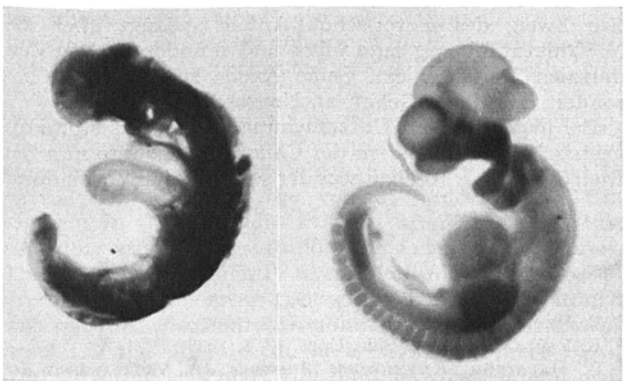

Fig. 6 Fig. 7

Fig. 1. 9 day embryo C57B1/6J. Control. Fig. 2. 10 day embryo C57B1/6J. Control. Fig. 3. 12 day embryo C57B1/6J. Control. Fig.4. 10 day embryo C57B1/6J. Experimental. Note arrested development. Fig. 5. 11 day embryo C57B1/6J. Experimental. Note arrested development. Fig. 6. 12 day embryo C57B1/6J. Experimental. Note arrested development. × 9.

Fig. 7. 10 day embryo Swiss albino. Control. Fig. 8. 12 day embryo Swiss albino. Control. Fig. 9. 10 day embryo Swiss albino. Experimental. Note open neurocoele in the brain and upper spinal region. Fig. 10. 11 day embryo Swiss albino. Experimental. Note exencephaly and tail indentation. Fig. 11. 10 day embryo Swiss albino. Experimental. Arrows point to tail indentation and to hematomata. Fig. 12. 10 day embryo Swiss albino. Experimental. Arrow points to indentation at tip of tail. × 9.

revealed that the C57 control embryos developed somewhat slower than the Swiss albino control embryos (Table I and II), as indicated by crown-rump length attained. On days 7 and 8 of gestation, the increase in size of C57 embryos was less than that of Swiss albino embryos (Table III, group 1 and 10, 2 and 11). On the 9th, 10th, and 11th days, the C57 and Swiss albino embryos did not differ significantly with respect to crown-rump length (Table III, group 3 and 12, group 4 and 13, 5 and 14), and by the 12th day, the 2 strains diverged again, and the Swiss albino embryos surpassed the C57 embryos in growth (Table III, group 6 and 15).

Discussion. The present data show an influence of the genetic background on the response of embryonic mice to the teratogenic action of trypan blue similar to that first reported for other agents by DAGG[2,3], FRASER[4,5], FRASER and FAINSTAT[6], LANDAUER[9], LANDAUER and BLISS[10], and RUGH, GRUPP and WOHLFROMM[11].

Although the C57B1/6J and Swiss albino strains differ in developmental rate, as indicated by differences in the crown-rump measurements, it is unlikely that differences in developmental timing operate to influence the distinct response of the embryos of the 2 strains to trypan blue. Treatment of Swiss albino mice as early as day 6, when embryos of this strain presumably resemble the stage of maturation characteristic for C57 mice at day 7, was never accompanied by the type of total developmental arrest that was displayed by the C57B1/6J embryos obtained from mothers treated on day 7.

The second point deserving comment concerns the observation that in Swiss albino mice, unlike C57B1/6J mice, the incidence of abnormalities diminishes with advancing embryonic age, so that on the 12th day of gestation the frequency of malformed embryos, obtained from Swiss albino mothers injected with trypan blue, decreased from 69.4% on day 10 to 25.4% on day 12 of gestational age.

Since the average litter size remained unchanged between days 10 and 12 of gestation (Table II), the decrease in incidence of abnormalities with advancing fetal age is, therefore, best explained by assuming that repair may be taking place during this period in the Swiss albino strain, while capacity for repair seems to be altogether lacking in C57B1/6J embryos. The possibility that differential capacity for repair of the embryos may be one of the genetic strain differences underlying their variability of response to an external teratogen deserves further study[12].

Zusammenfassung. Die Wirkung des Farbstoffes Trypanblau auf die embryonale Entwicklung von 2 Mäusestämmen, des C57B1/6 Stammes und des Swiss-Albino-Stammes, wurde untersucht und die Ergebnisse wurden miteinander verglichen. Embryonen der beiden Stämme reagierten in verschiedener Weise auf Trypanblau. Die Injektion des Farbstoffes führte zum vollständigen Entwicklungsstillstand aller C57B1/6-Embryonen, während bei Swiss-Albino-Embryonen hauptsächlich Kopf-, Gehirn- und Schwanzabnormitäten auftraten. Bei Swiss-Embryonen nahm die Schwere der Abnormität mit fortschreitendem Alter der Embryos beträchtlich ab, obwohl die Anzahl pro Wurf unverändert blieb. Keine Veränderung in der Zahl der Abnormitäten wurde in älteren C57B1/6-Embryonen beobachtet. Die verschiedenartige Reaktion der beiden Stämme auf Trypanblau ist vielleicht darauf zurückzuführen, dass genetisch bedingte Unterschiede in der Fähigkeit, zerstörtes Gewebe während der embryonalen Entwicklung zu reparieren, vorhanden sind.

M. HAMBURGH and VERONICA CALLAHAN[13]

Department of Biology, City College of New York (N.Y. 10031, USA), 2nd January 1967.

[9] W. LANDAUER, J. exp. Zool. *105*, 145 (1947).
[10] W. LANDAUER and C. I. BLISS, J. exp. Zool. *102*, 1 (1946).
[11] R. RUGH, E. GRUPP and M. WOHLFROMM, Proc. Soc. exp. Biol. Med. *106*, 219 (1961).
[12] Supported by Training Grant No. 1T1 HD 116-01 of the National Institute of Child Health and Human Development.
[13] Submitted in partial fulfillment of the requirements for the degree of Master of Arts in the Graduate Division of the College of Liberal Arts and Science of the City College of the City University of New York.

Der Ursprung des dorsalen Herznervs der Lithobiiden (*Chilopoda*)

Die Chilopoden besitzen einen Nerv, der das Herz dorsal begleitet und innerviert. Er wurde deshalb als «dorsaler Herznerv» beschrieben[1]. Eine Verbindung dieses Nervs mit dem Zentralnervensystem konnte durch diese histologischen Untersuchungen ebensowenig wie später durch embryologische Beobachtungen[2] festgestellt werden. Es wurde daher gefolgert, dass der dorsale Herznerv ein «selbständiges System» bilde[3]. Vom Standpunkt der vergleichenden Anatomie aus ist diese Annahme sehr unwahrscheinlich. Deshalb war die Möglichkeit ins Auge gefasst worden, dass der Herznerv wenigstens über segmentale Nerven des Bauchmarks mit dem Zentralnervensystem in Verbindung stehen könnte[4], wie es von einigen Cheliceraten und Insekten bekannt ist. Diese Vermutung wurde schliesslich bestätigt[5]. Der caudale Nerv eines jeden Ganglions gabelt sich unmittelbar nach seinem Austritt. Ein Ast zieht an die Dorsalseite und gibt einen sensorischen Anteil an das Tergum, einen motorischen vor allem an die dorsale Muskulatur ab. Ein Zweig des motorischen Anteils gelangt über die Muskulatur hinweg zum Herz und mündet dort in den dorsalen Herznerv ein. Seine Axone zeichnen sich besonders durch den Gehalt an Neurosekret aus[5].

Bei histologischen Untersuchungen über das stomatogastrische Nervensystem der Chilopoden konnte nun bei *Lithobius piceus* Koch ausser der segmentalen Verbindung

[1] C. HERBST, Zoologica, Stuttg. *3/9*, 1 (1891).
[2] R. HEYMONS, Zoologica, Stuttg. *33*, 1 (1901).
[3] K. FAHLANDER, Zool. Bidr. Upps. *17*, 1 (1938),
[4] B. HANSTRÖM, *Vergleichende Anatomie des Nervensystems der wirbellosen Tiere* (Springer, Berlin 1928).
[5] H. SCHEFFEL, Zool. Jb. *79*, 529 (1961).