

Tolerance 1952-4

Publication/Creation

1952-1954

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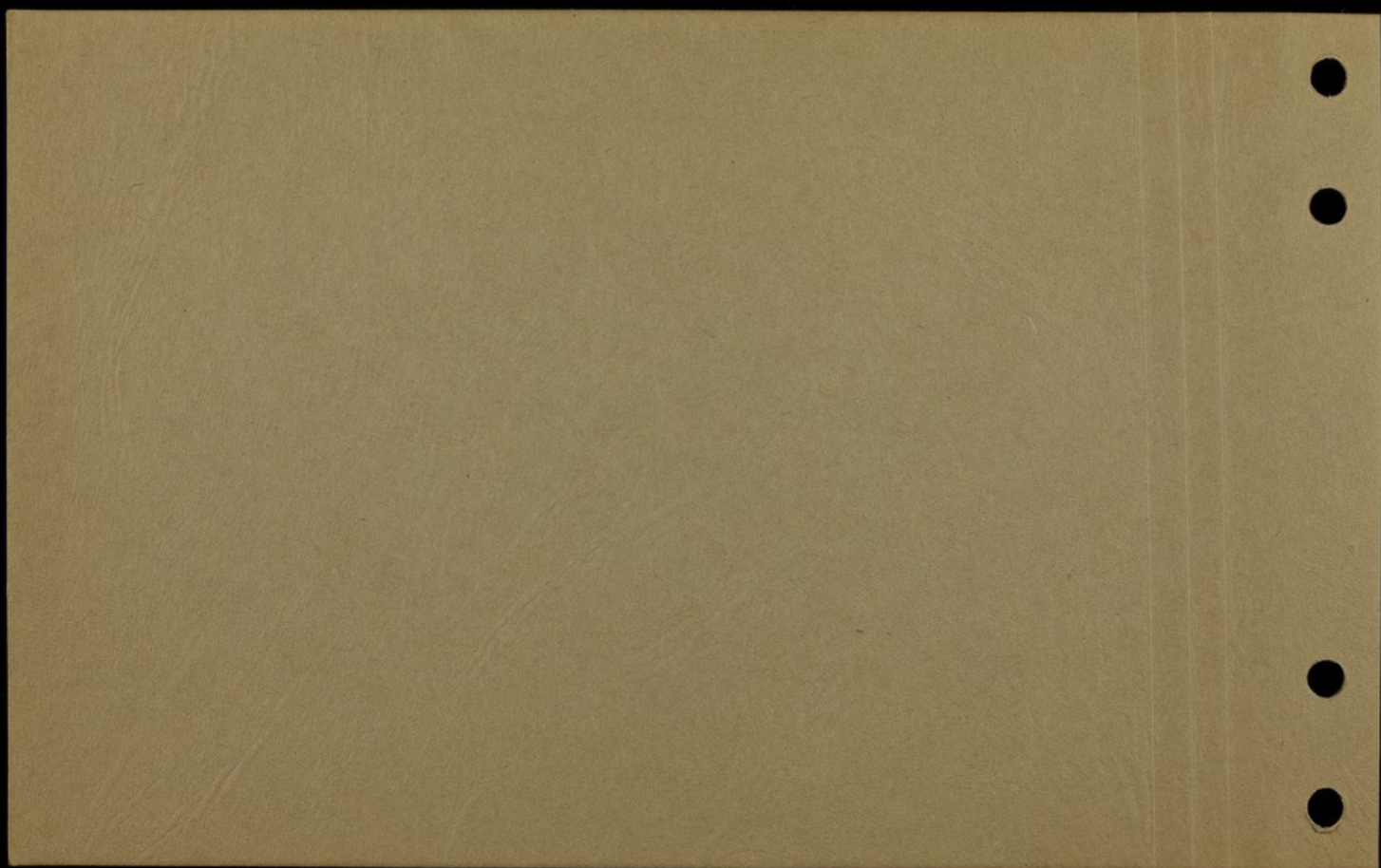
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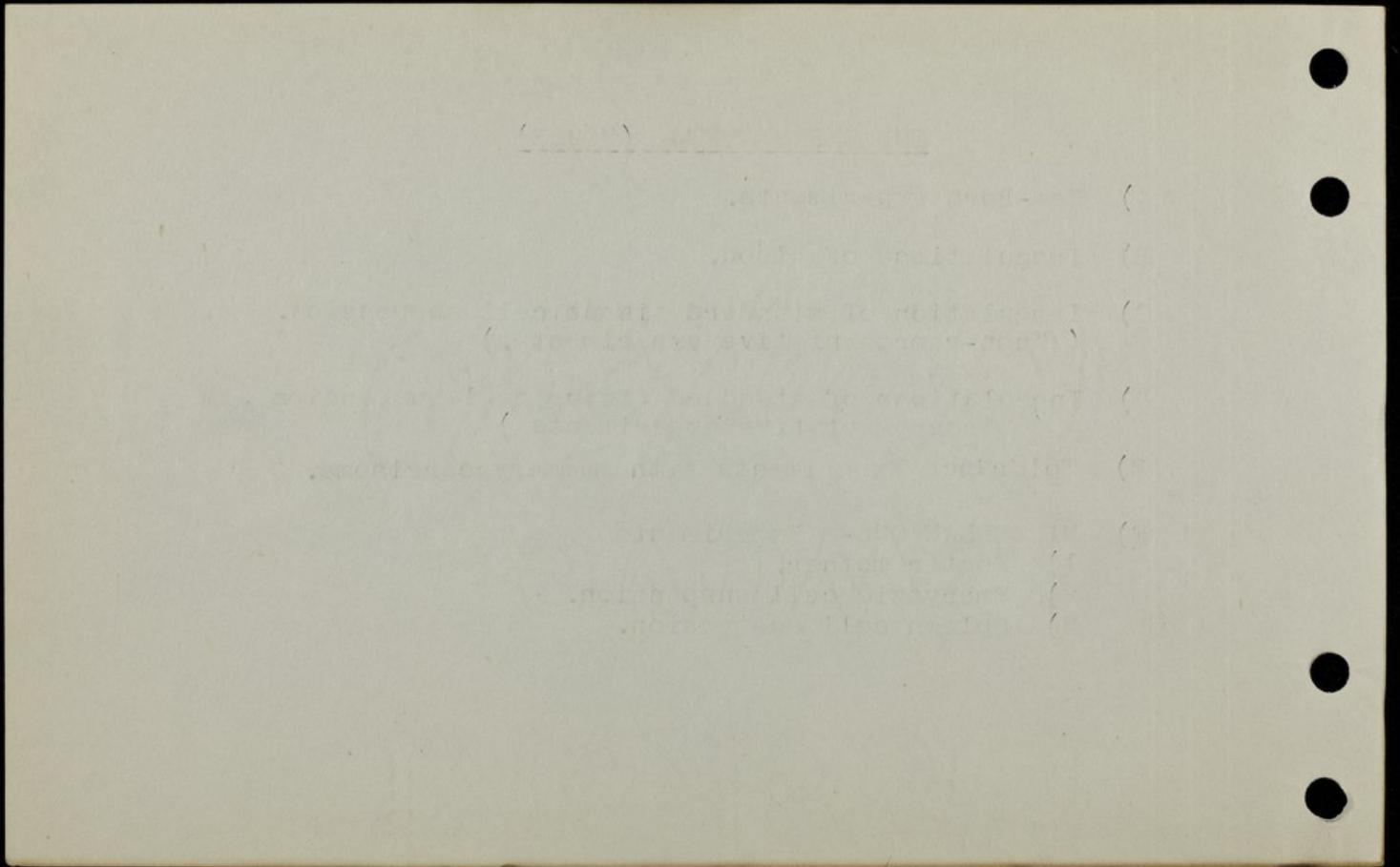


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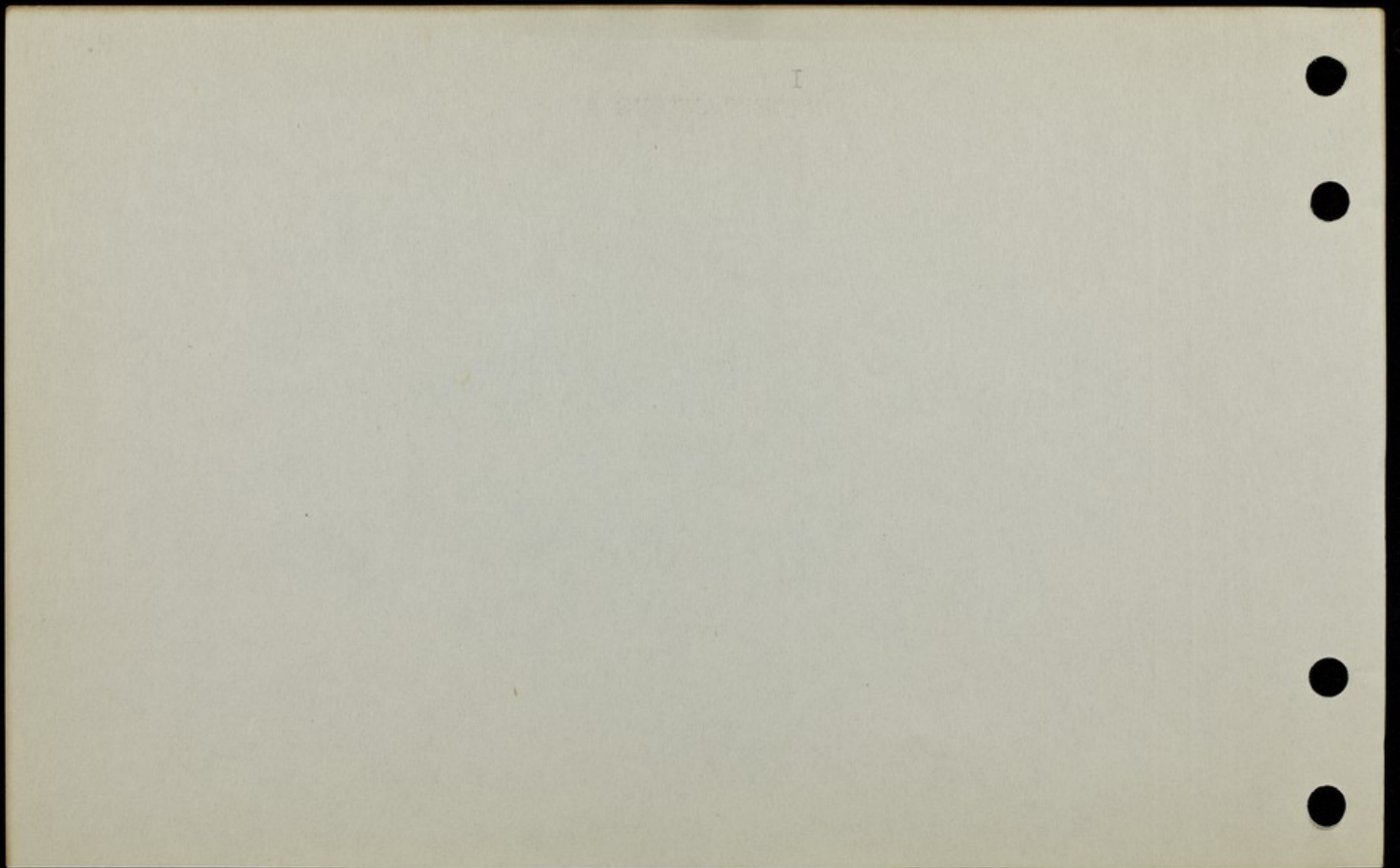
EMB EXPERIMENTS (MOUSE)

- A) New-Born experiments.
- B) Inoculations of blood.
- C) Inoculation of standard tissue cell suspension.
("non-representative experiments.")
- D) Inoculation of standard tissue cell suspension .
("representative" experiments)
- E) Tolerance Experiments with mammary carcinoma.
- F) MISCELLANEOUS- Experiments
 - 1) Foster mother.
 - 2) Embryonic cell suspension.
 - 3) Spleen cell suspension.



F.

I
MSCELLANEOUS



5 DEC 1953 Donor: Exactly as for EMB-147 - note SPLEEN only.

Recipients: Foetuses of cba female with exactly the same credentials as for EMB-147. R notch, 39 g.

8 embryos seen, but only 7 could be injected. Visualisation fairly good. Age of embryos 16-17 days. 0.01 cc - this representing about 1/20 of an adult spleen. 5 of the 7 injections are considered to have been reliable.

8 DEC 1953 Healthy litter born at about mid-day this day: 6 live, 1 dead. Age at time of injection therefore a little more than 16 days. A very hopeful experiment.

22 DEC 1953 EMB mother found dead this a.m., presumably having died overnight. Transfer the litter of 7 to 7A2/P, which had a seventh litter on 11 th December); and transfer its litter to 7A1/P, which has just had a 6th litter of only 3, which were destroyed.

16 JAN 1954 The entire litter successfully weaned. Note, therefore, that for two weeks it has been suckled by a mother of the graft donor strain.

20 JAN 1954 Test-operation.

Donor: Stock 8 A-line male. Standard grafts - possibly slightly smaller than usual. Operate all 7 mice - RHS opns.

31 JAN 1954 (11) Primary inspection.

4/7 grafts show normal b.d. picture.
1/7 has high degree of survival but b.d. in progress.
2/7 100% survival.

1 FEB 1954 (12) ~~3/7~~ now show completed b.d.
1/7 now has advanced b.d., but still a small patch of survival.
1/7 is predominantly intact, but somewhat swollen and with
very slight superficial scabbing. There is just a possibility that there are new
hairs, though it does seem rather too early.

2 FEB 1954 (13) Only one graft is now showing survival. This has some central
scabbing and prognosis is very poor.

4 FEB 1954 (14) Scabbing of the last surviving graft is now just complete.

Survival scores: 12 and 14 days - barely significant.

EXPERIMENT CONCLUDED

5 DEC 1953 Donor: Remove WHOLE SPLEEN from male A-line Stock 8 mouse, chop up with scissors and remove the supernatant with its cells and cell clumps at frequent intervals by passing it through 26 Agla syringe needle. Chop up the whole of the spleen in this way. Spin, discard supernatant, and resuspend the cells in a small quantity of Ringer. Suspension thus obtained is more dilute than usual, and it probably contains more single cells since the spleen on its own chopped up very easily. It is easy to keep it homogenous by light shaking, and there is little evidence of sedimentation. A good suspension.

Recipients: Foetuses of cba Stock 5 female, pregnant by Stock 2 male, S.D., second preg., no notch, 39 g.

Inject 2 + 5 embryos - very near term. 0.01 cc - this represents a little less than 1/20 of an adult spleen. Visualisation very good and injections are reliably intra-embryonic.

07 DEC 1953 Healthy litter of 7 found to-day, but most probably born yesterday. One found ^{dead} later in day - rest are being suckled.

20 JAN 1954 Test-operation.

Donor: Stock 8 A-line male. ~~Standard~~ Grafts generally slightly smaller than usual. Operate all 6 mice - RHS.

31 JAN 1954 (11) Primary inspection.

2/6 grafts with typical b.d.

2/6 grafts show high degree of survival, but are inflamed and with some weakness.

2/6 grafts show 100% survival, but prognosis not too favourable.

1 FEB 1954 (12) 2/6 have fairly advanced scabbing, but there is still some survival.

2/6 have predominantly viable epithelium, but there is slight scabbing in both.

3 FEB 1954 (14) Only 2 grafts show any degree of survival: one with only slight, the other with a high degree of survival.

5 FEB 1954 (16) Only one graft is now going: this has almost complete epithelial survival though it is smallish, bald and shiny, with a rather scurfy cuticle.

10 FEB 1954 (21) Small graft still in position and appears to be robustly epithelialised, but no sign of hair-growth.

This mouse has just had a litter - kill this.

17 FEB 1954 Reoperation. (28)

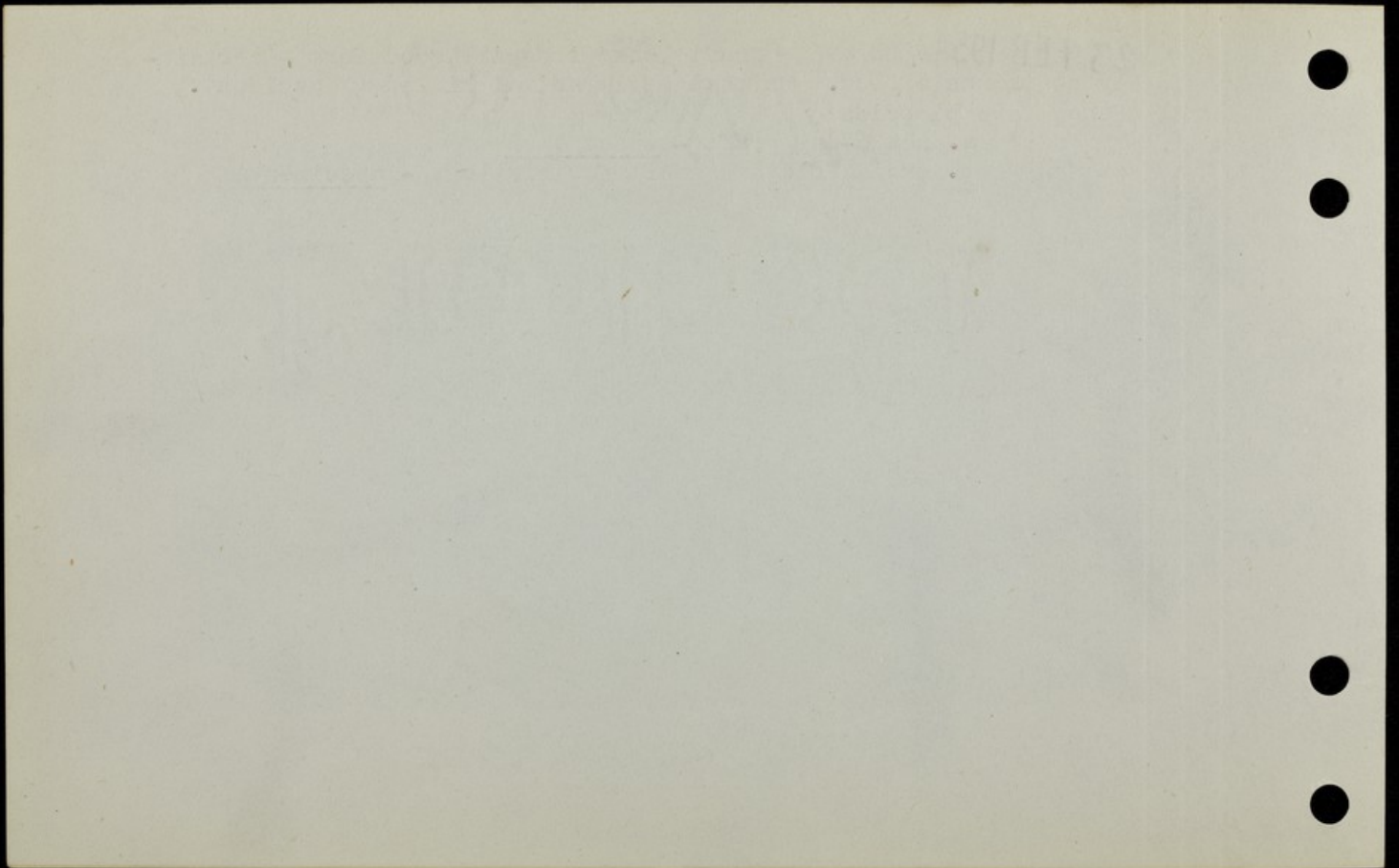
A very small graft appears to be in position and quite robustly epithelialised, but without signs of incipient hair-growth.

To ascertain reactivity of host, regraft on **LHS**. Donor: Stock 7 A-line male.

Inspect at 6 days.

23 FEB 1954 (28 + 6) Graft looks in quite good form - certainly no immune effect. Unfortunately animal killed by accident. Hence take biopsies

- a. new 6-day graft - specimen B
- b. excise old RTW graft for histology - specimen A



INOCULATION OF F0TELA MICE (CBA to A) WITH ADULT
BLOOD LEUCOCYTE CONCENTRATE

EMB-163

25 JAN 1954 DONOR CELLS:- Pooled leucocyte concentrate from 16 CBA immune donors: SEE PT-37 this day. (The leucocytes were prepared from the buffy coat of gently spun citrated whole blood, and should be perfectly viable. About 50% by bulk of the cells were red cells.)

RECIPIENT:- Primiparous A-line female of litter 8A5/1, preg. by litter mate (first pregnancy). No mobilization.

Find five foetuses, circa 18-day. One injected sub-integument; two other intraembryonic; two others, from faulty orientation, were probably but not certainly intraembryonic. 0.01 ml.

27 JAN 1954 4 (+ 1 stillbirth) born early this a.m. (afterbirth seen 9 am & foetuses still sticky). Hence put birth at 6 a.m., or 36-40 hrs after operation.

29 JAN 1954 3/4 have dark internals blood stains that suggest successful injection - not intraperitoneal but dorsally in anterior part of chest. 1/4 is very feeble & backward: will not live. Unfortunately, this is one of the 3 with blood stains.

30 JAN 1954 As expected, the fourth mouse died. Others healthy.

27 MAR 1954 Test-operation.

Donor: 7C4/P male. Standard grafts. Graft to RHS of all 3 mice.

15 APR 1954 (9) 1/3 with peeling epithelium - destroy.
1/3 with 100% survival but perceptible weakness.
Eosin. 1/3 100% survival, some perceptible pigmentation over
whole graft. SUPER.

16 APR 1954 (10) 1/2 with about 50% survival - scabbing in progress.
1/2 still 100%, but small psoterior scab which may be non-specific. Graft pigmented, and slightly puffy.

17 APR 1954 (11) 1/2 still some, but slight, survival.
1/2 little change - almost complete survival.

18 APR 1954 (12) 1/2 breakdown complete.
Survivor: Almost 100%, but slight marginal scabb&ing and puffiness. Pigmentation.

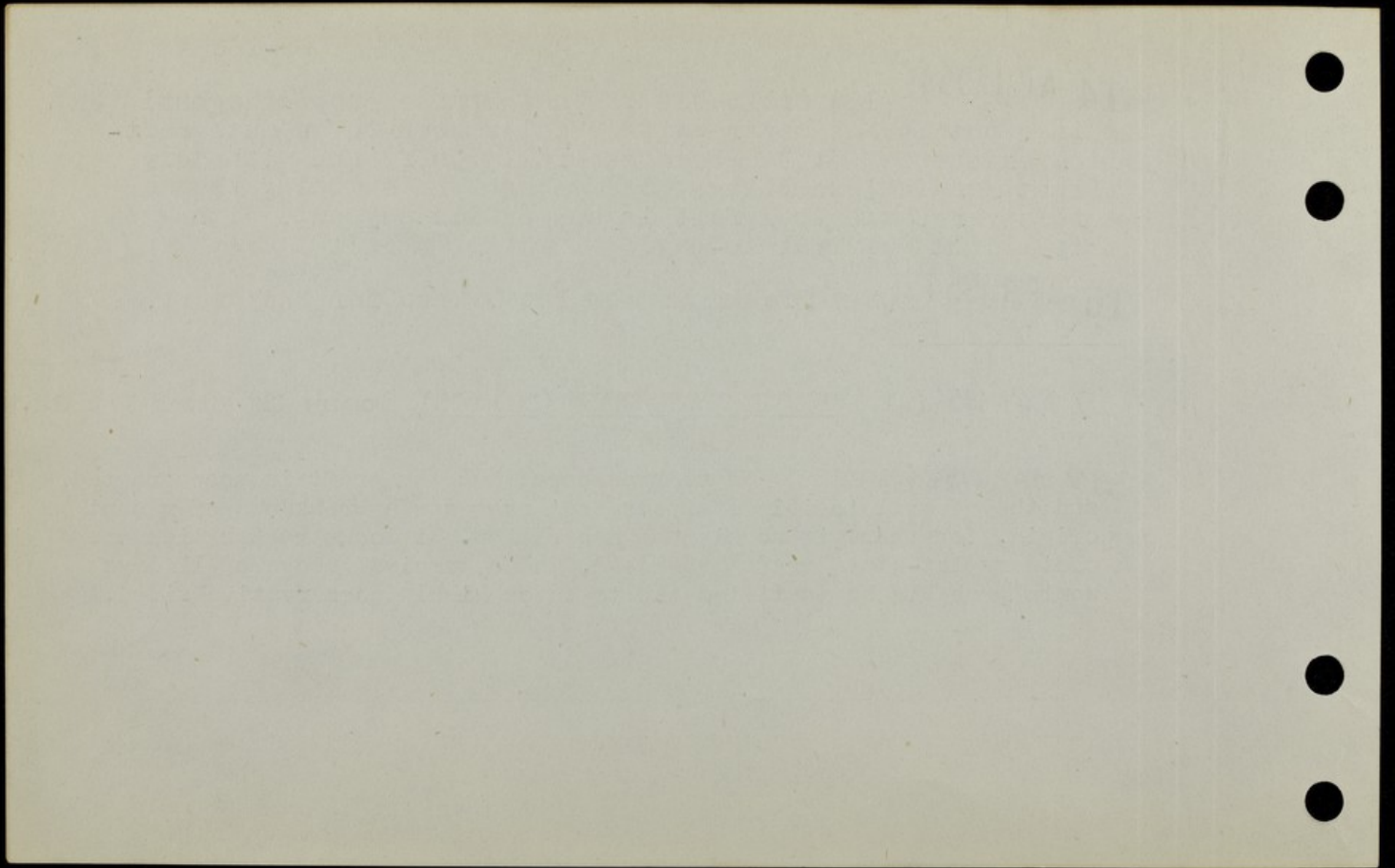
19 APR 1954 (13) Not much further detariotation: Sparse hairs have erupted.

14 APR 1954 (18) Graft had grown a sparse crop of agouti hairs, but it has never quite settled down to an autograft-like existence. It has been generally puffy and with very slight marginal scabbing. 3 days ago the scabbing became more severe, and the graft is now on its way out, with only slight survival to-day.

16 APR 1954 (20) Breakdown complete. Keep for 2nd graft.
Score: 20 days.

7 MAY 1954 (41) SECOND STAGE GRAFT (LHS OPN). Donor: CBA Stock XV male.

19 MAY 1954 (41 + 12) Total breakdown; but the graft is uncontracted, and the general 'clinical appearance' suggests a breakdown of recent origin, for there is no sign of maceration. It looks rather like an ordinary 1st-stage graft breakdown, and a survival score of **11 days** would probably be justified (10 to be on highly conservative side).



INOCULATION OF FOETAL MICE (A to cba) WITH EMBRYONIC
CELL SUSPENSION THROUGH BODY WALL.

EMB-85

5 MAR 1953 Donor tissue: Exactly as for EMB-83.

Recipients: Foetuses of female (o-parous) of litter 305/3, b.24 Dec., pregnant by 5C1/p, special diet since 3 March.

Only 3 foetuses seen and injected. Very near term. Injections subcutaneous, but one probably injected into head region. 0.01 cc each.

6 MAR 1953 Litter of 3 born at 4 p.m. - just over a day after ops. 3

21 APR 1953 Test-operation.

Donor: Remove standard body-skin pinch grafts from female of litter 443/4, b.5 March. Scrape.

Recipients: Transplant one graft to RHS of each mouse - usual method.

1. F (R) 19 g.
2. F (L) 18 g.
3. M 22 g.

30 APR 1953 (9). First Inspection.

1. Healing exemplary. Cuticle came away cleanly to reveal dry roof which is firmly epithelialized. There is very little indication of a reaction. Redress.
2. Healing O.K. Graft roof is moist and obviously without epithelium; it scabbed on exposure. KILL.
3. Healing O.K. except for small fault dorsally. Graft is thin and fully epithelialized. Redress.

2 MAY 1953 (11)

1. Roof dry, pink and epithelialised but rather crinkled and slightly reddened at one or two points - this may be due to the fact that no tulle was applied before replastering at the last inspection. The dorsal fault ~~is healed~~ appears to be healing. Graft predominantly alive, but general appearance not quite "super". Germolene.
3. Roof pink and dry - peel off what must surely be thin cuticle and not epidermis, revealing epithelialised surface. Graft appears to be showing complete survival - too early to say whether "super".

4 MAY 1953 (13)

1. Graft considerably contracted and with haemorrhagic scabby patches ventrally and dorsally, **but nevertheless central survival** - it is quite firmly epithelialised in this area. Prognosis however poor.
Photograph (colour) ✓
3. Central haemorrhagic and somewhat scabby patch, but graft predominantly surviving. Some contraction. Epidermis looks healthier than at 11 days

and it is not inconceivable that graft may recover.
Photograph. (Carrick) ✓

EMB-85

5 MAR 1953 (14)

1. The dorsal and ventral scabs have linked and spread. Further contraction. The graft is now on the way out but a patch of surviving epithelium is still in place posteriorly.
3. Some further contraction has taken place but not as prominently as in 1. The area of the central scab has increased slightly but there is a wide tyre os clearly surviving and not altogether unhealthy looking graft epithelium.

6 MAR 1953 (15)

1. Scabbing complete. This graft can be safely scored fora survival time of 14 days. This is significant prolongation.
3. No change. Graft predominantly surviving.

8 MAY 1953 (17)

3. Condition of graft quite unchanged - scab seems to be healing and area is firmly epithelialised with marginal delicacy. No hair-growth. Graft appears to be surviving.

11 MAY 1953 (20)

3. No change. Graft has not undergone further contraction, is fully if ~~slightly~~ delicately epithelialised, ~~and~~ scar healing, no hairs. **Graft appears to be surviving.**



16 MAY 1953

(25) 3. During the last 5 days steady contraction has taken place and the graft has gradually dwindled into a scar. This graft has shown a reaction from the very start and it came probably to completion shortly after the 20th day.

INTRA-EMBRYONIC INOCULATION OF EMBRYOS (CBA to A) WITH
FOETAL CELLS: THROUGH BODY WALL.

EMB-68

22 DEC 1952 DONOR TISSUE: Strong whole embryo micrograft brei (13½ day) of same sample as that used for EMB-62 q.v. this day.

RECIPIENT: The last female of the litter 3A2/4 which has been on supplementary diet (milk, occasional cheese & bacon, cod-liver oil, grape nuts) since Nov, 27. Pregnant by litter mate; in perfect condition & nesting well. Destroy the male .

Shave midventral band of skin; wipe off soap (no spirit) and smear with germolene. Make fairly long incision and mobilize skin on either side, taking great pains not to interfere with mammary vessels. Four embryos seen most distinctly through body wall: they look near term (say 18 days). Four of these were successfully injected intraembryonically (just possible that one of these not successful, i.e. intrauterine). Sharp needle. Weight was 33 g, excellent for primiparous mouse. After injection do particularly close triple skin sutures and finish with sulphadiazine. Ringer was used to keep the body wall moist, but the mouse was not soused in Ringer..gauzes damp but not soaking with excess fluid. Anaesthesia by full nembutal dose.

Mouse recovered and was moving actively about within an hour of operation. Looks in good shape.

23 DEC 1952 10 a.m. Mouse nesting perfectly, & very alert & lively; but one of the more anterior abdominal skin stiches has come adrift, & this may prejudice success of operation. Must be prevented in future.

24 DEC 1952 10 a.m. 6 pink and lively young born; look normal, but not inspected closely. Probably the whole litter.

Notes: The nest is deep, as with EMB-64, by the addition of further hay on top of existing nest. Diluted milk is being offered alternatively to water by having two bottles.

25 DEC 1952 Only 5 young counted; was this the original number anyway?

13 FEB 1953 Test operation.

Donor tissue: Standard pinch grafts (trimmed) from male of litter 3C4/3, b.8 Nov.

Recipients about 7 weeks old. Note weights which are considerable for this age (supp. diet of mother).

1. (R) M 27g. Ops. O.K.
2. (no) M 24 g. Ops. O.K.
3. (R) F 21 g. Ops. O.K.
4. (L) F 21 g. Ops. O.K.
5. (no) F 22 g. Ops. O.K.

21 FEB 1953 (8)

1. Healing faultless. Pick off cuticle - roof dry, but graft generally inflamed, especially ventrally. Typical breakdown picture. Rebandage.
2. Healing faultless. Cuticle off cleanly. Apart from a few scattered weakish points this graft is in good shape. Rebandage.
3. Healing faultless. Cuticle off cleanly except for a largish

patch where agouti hairs remain attached. Graft looks in good form except for two small foci of inflammation. Rebandage.

4. Graft looks in very good shape, best so far. Some cuticle adheres and is left in position. Rebandaged.
5. Healing faultless. Cuticle off cleanly. Roof definitely epithelialised with no trace of haemorrhage, but graft is rather white and slightly puffy. Rebandage.

23 FEB 1953 (10)

1. Graft obviously dead, with yellowish discoloration and marginal ingrowth taking place. Scabbing after some hours of exposure. Kill.
2. Graft uncontracted and dry-roofed, and seems to be covered by robust epithelium. The latter is matt except in dorsal 1/3 where the surface is redder and the epithelium rather dead-looking. No cuticle to pull off. Graft on the whole promising. Rebandage.
3. No cuticle, hairs as noted at (8) still attached. Pull out and with them the dead epidermis. Graft very white, epidermis peels off easily. Breakdown complete. Scabbing after a few hours of exposure. Kill.
4. Epidermis discoloured, looks dead and peels off easily. Breakdown complete. Scabbing after a few hours exposure. Kill.
5. Very thin and scaly cuticle, roof dry and epithelialised. Graft rather thicker than host skin but quite supple, greyish coloura-

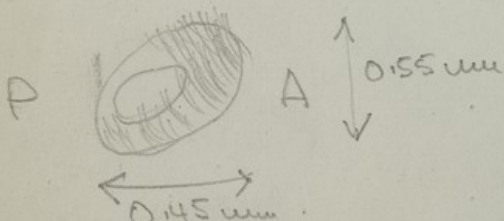
tion may be due to pigmentation. Very hopeful. Rebandage.

2 and 5 therefore remain as possible super-mice.

26 FEB 1953 (13)

2. Graft has a bad dorsal scab and yellow discolouration. Epithelium peels off easily to reveal naked graft dermis. Scabbing on exposure. Kill.
5. Graft uncontracted, dry surface with scaly cuticle, epidermis pigmented (deep grey), erupted pigmented hairs just visible to the naked eye anteriorly. Graft supple. Super-mouse?

2 MAR 1953 (17)



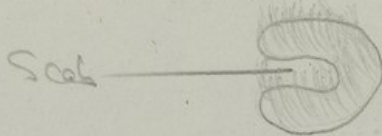
By late afternoon of the 13th day (i.e. a few hours after removal of bandage) a slight weakness was spotted in the posterior half of the graft. This weakness had developed into a fairly large sear by the 14th day and has been watched since with some anxiety. At the same time the anterior part of the graft seemed healthily pigmented and growing a good crop of agouti hairs.

Careful examination on this day (after clipping of the surrounding host hairs which are in an active phase): A dry and probably 'healing' scar area occupies the central and somewhat posterior part of the graft. The rest of the graft is healthily pigmented, largely covered by agouti hairs, and is obviously surviving. Hairs are particularly prominent anteriorly. There has undoubtedly been some contraction but L.B. remembers that original graft was rather

smaller than usual. Hence contraction by no means severe. EMB-68

General impression: Graft has gone through a specific reaction but is holding its own and may well make a complete recovery. This would reenact a similar condition noted in free-martins by PBM and REB.

5 MAR 1953 (20) Skin around graft in phase of active hair-growth, clip away hairs and examine graft. The scab noted before is still there, and is posterior and central in location. It is surrounded by a horse-shoe of deeply pigmented epithelium which is producing a dense crop of long agouti hairs.



6 MAR 1953 Test operation for B-line graft.

Donor: Remove one graft from flank of female of litter 3B3/1, b.24 Sept. Trim. transplant to scissors-clipped bed on LHS of this animal. Ops. O.K. Primary agouti graft was covered with small piece of tulle to prevent adhesion of hairs to plaster bandage. Inspect at 8 days.

This expmt. should help to establish the specificity of desensitisation.

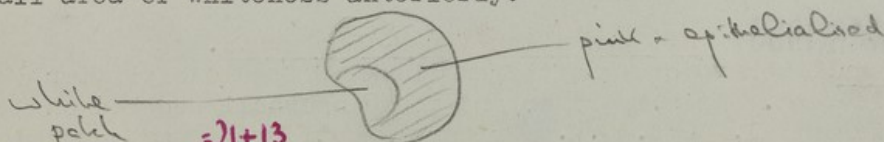
14 MAR 1953(21 + 8). The LHS B-line graft perfectly healed. The entire surface layer of cuticle & hairs came off in one piece, discovering a

glistening moist surface, patchily haemorrhagic, which browned in air. Breakdown far advanced, but possibly some survival of epithelium at periphery.

The RHS original CBA graft had almost certainly grown (typical agouti) hairs since 21 days. These plucked away easily, and by washing the graft surface, note that graft is raised and a bit damp and pulpy. Some survival of epithelium over the graft is certain, but it is equally certain that the graft is going through a thoroughly bad time.

Replaster

17 MAR 1953 (32) Graft appears soundly epithelialised, rather pink (possibly slightly more so than in a healthy graft). One small area of whiteness anteriorly.



19 MAR 1953 (34) Graft much as on the 17th, and covered by a well defined and quite robust cuticle. Incisive outline. Colour generally pinkish, but with whitish patches. Graft obviously epithelialised, and appears to be surviving. &

Apply germolene, do not rebandage.

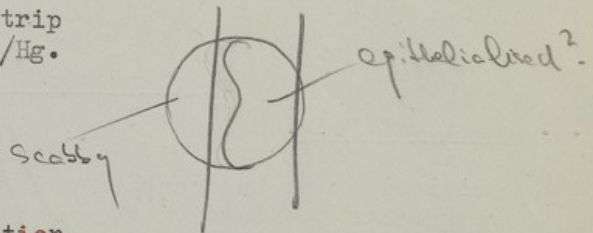
Some time after exposure a patch of scabbing has appeared anteriorly - watch carefully.

EMB-68

The scabbing has increase anteriorly so that nearly the whole of the anterior half has scabbed. The rest is certainly epithelialised and shows no sign of necrosis. This area roughly approximates with that part of the graft which had previously shown active hair-growth.

To establish whether there is some surviving epithelium in order to clinch the specificity problem, biopsy but keep animal alive so that a further cba graft can be grafted in due course.

Specimen EMB-68.1. Cut out strip as shown in diagram. Formol/Hg.



30 MAR 1953 Second test-operation.

Donor: Standard body-skin pinch graft from male of litter 4C2/2, b.6 Feb. Panniculus scraped off. Bed prepared in usual manner (scissor-clipped). **LHS.**
The site of the old graft has healed completely.

4 APR 1953 This mouse is early-pregnant, by either 4A4/3 or 4C2/2 (see EMB-62).

7 APR 1953 (8) Healing O.K. except for slight fault at dorsal margin. Ghost off cleanly. Graft moist dorsally, rather white with haemorrhagic flecks. Definitely some surviving epithelium - hence

no immune effect. Rebandage.

10 APR 1953 (11) Most of graft has scabbed. There is a small semi-circle of what might be sound epithelium. Germolene only.

11 APR 1953 (12) Graft has scabbed completely. Kill.

Breakdown has followed a more or less normal course, and there have been no symptoms of an immune effect. The last-mentioned fact is exceedingly puzzling and awaits an explanation.

Histological report.

68.5. Survival fair.

It is impossible to give an accurate score since about half of the original graft had scabbed quite early on and this specimen merely represents the surviving remnant. But there can be no question that graft epithelium is present, and this is fortunately verifiable since melanocyte processes can be seen in the epithelium.

Once again, this graft proves the specificity of desensitisation. Note that this graft went through a pretty severe reaction quite early on (14th day), recovered after losing about half of it, and then went through another phase of reaction after the transplantation of the B-line graft (21 + 8) and again recovered.

27 JAN 1953

Breeding-pair 202/6.3+4, b.12Nov. 1st litter born during night of 25/26, and hence some 36 hours old when transferred to 5A3/P to be fostered. (7 young).

The A-line litter of 8 was in turn given to 202/6.3+4 to suckle.

9 FEB 1953

Cba foster-mother dead - cause unknown. Destroy male also and transfer litter (A) to new foster-mother 305/2, b.11 Nov.

31 MAR 1953

Test operation.

Donor: Male of litter 4A4/3, b.12 Feb. Remove standard body-skin pinch grafts and trim.

Recipients;

1. M (R) 23 g.
2. M (L) 24 g.
3. F (R) 19 g.
4. F (L) 20 g.
5. F (no) 20 g.
6. F (both) 20 g.
7. F (2R) 20 g.

11 APR 1953 (11)

1. Typical breakdown. Moist and haemorrhagic. Scabbing on exposure. Kill.
2. Anatomically intact epidermis peels off easily, revealing moist, speckled dermis. Scabbing over peeled surface. No survival. Kill.
3. Same as for 2. Kill.
4. Epidermis anatomically intact, dry-roofed, no sign of haemorrhage, but very white. Epidermis peels off easily. Scabbing. Kill.
5. Typical breakdown - graft de-epithelialised apart from small patch dorsally. Scabbing. Kill.
6. Same as for 2. Kill.
7. Same as for 5. Kill.

Note: This is a typical MST breakdown picture. Compare with EMB*71.
Note that this litter was about 36 hours old when transferred.

27 JAN 1953

4A1/2.3+4 = 5A3/P, b.10 Oct.

Litter born round about mid-day = 5A3/2.1-8

Within 2 hours of birth this litter was transferred to breeding-pair 2C2/6.3+4 which had given birth to their own litter some 36 hours before.

The cba litter of 7 was in turn given to 5A3/P to suckle.

9 FEB 1953

The A litter transferred to new foster-mother 3C5/2, b. 11 Nov., on death of 2C2/6.3. This mouse was born on the 11 Nov. and was nursing a litter of 4 of same age.

13 FEB 1953

2nd foster-mother also dead. 4 young given to a B-line female to suckle, the other 4 to aa A-line female belonging to M.W., i.e. the litter is no longer being suckled by a "donor strain" female. This should be immaterial at this stage. The litter as a whole is rather backward, obviously due to the frequent chopping and changing of foster-mothers and some neglect.

30 MAR 1953 Test operation.

Donor: Remove standard pinch grafts (body-skin) from male of litter 4C2/2, b.6 Feb. Scrape off panniculus in usual manner.

Recipients:

1. F.(R) 19 g. Opn. O.k.
2. F.(L) 19 g. Opn. O.K.
3. F.(no) 18 g. Opn. O.K.
4. F.(both) 18 g. Opn. O.K.
5. F.(2R) 19 g. Opn. O.K.
6. F.(2L) 19 g. Opn. O.K.
7. M. (R) 21 g. Opn. O.K.
8. M. (L) 21 g. Opn. O.K.

Operations RHS. Scissor-clipped bed.

10 APR 1953 (1D - MST)

1. Graft has healed in well. Cuticle very adherent, and peels off to reveal moist graft roof which has almost certainly some surviving epithelium. On drying in air general appearance deteriorates with some scabbing, but still good reason to believe in some survival. Rebandage.
2. Healing faultless. Roof white and very moist, with faint haemorrhagic mottling. Complete scabbing on drying in air. No survival. **Kill.**
3. Healing faultless. Roof rather white and moist, but no scabbing or deterioration after exposure to air, colour on the whole pretty healthy, and graft epithelium is obviously **fully surviving.** Rebandage.
4. Same as for 3.

2

- EMB-71
5. Healing faultless. Cuticle off very cleanly. Graft roof moist, suspiciously white and with dorsal haemorrhagic patches, but almost certainly some surviving epithelium. Rebandage.
 6. Healing faultless. Moist roof on removal of cuticle. Scabbing after short exposure. No survival. Kill.
 7. Healing faultless. Roof dry, but with yellowish tinge. Epidermis peels away easily as a sheet. Scabbing on exposure. No survival. Kill.
 8. Healing faultless. Roof slightly damp with two haemorrhagic patches, but undoubtedly high degree of survival. Rebandage.

Total: 3 with high degree of survival, 2 with some survival, 3 with no survival. This would appear to be a significant result and may justify repetition of this experiment. Note that half the litter had a somewhat different history in suckling.

11 APR 1953 (12)

1. Graft slightly swollen, moist, and with patches of abnormal translucency. Scabbing on exposure. No survival. Kill.
3. Graft surface dry and firm. Very slight suspicion of mottling and translucency. No scabbing on exposure. Germolene only.
4. Graft superficially O.K. but epidermis peels easily. Scabbing on exposure. No survival. Kill.
5. Colour yellowish. Epidermis peels easily. Scabbing on exposure. Kill.
8. As for 4. No survival. Kill.

13 APR 1953 (14)

3. Graft in place, central region recessed and scabbed but marginal annulus of surviving epithelium still present. Overgrowth rather

unlikely as graft margin is pretty incisive. Some contraction.

14 APR 1953 (15)

3. Scabbing complete. Kill.

Breakdown therefore 14-15 days. This is ceratinly significant and justifies repitionn of the expmt.

20 APR 1953 This litter of 8 is 5A2/5.1-8, and was transferred to 2C2/6.1+2 at a maximum of 11 hours after birth.

19 JUN 1953 Test-operation.

Litter about 7 weeks old.

Donor: Inbred cba mouse from special diet (EMB) stock-pot, but exact litter number not known. (Probably 4C3/1, b.5 March).

Kill mouse and remove 8 standard body-skin pinch grafts. Remove panniculus by scraping. Skin very **active**.

Recipients: Transplant one graft to each member of the litter. Usual RHS scissors-clipped bed.

1. F (R) 20 g.
2. F (L) 18 g.
3. F (no) 20 g.
4. F (both) 20 g.
5. F (2R) 15 g.
6. M (R) 22 g.
7. M (L) 22 g.
8. M (no) 22 g.

All opns. O.K.

19 JUN 1953 (10)

Anaesthetise all mice in this group with a sublethal dose of nembutal and inspect all grafts together. Final judgment passed after allowing exposure to air for about 15 minutes.

3/8 are clearly without surviving epithelium, the grafts having scabbed over completely.

1/8 has probably pretty good survival, a little more than 50%, but some parts have scabbed.

4/8 can have no more than the slightest trace-survival. The grafts have scabbed over almost completely, and there is only the slightest doubt at one or two marginal points.

This result is in complete agreement with the control series at the MST.

20 JUN 1953

The graft which had good survival yesterday has now scabbed completely.

20 JUN 1953

20 JUN 20 APR 1953 This litter of 7 is the 4th from 2C2/6.1+2, and was transferred 14 hours after birth to 5A2/P.

8 JUN 1953 Test operation.

Litter about 7 weeks old.

Donor: Kill young male from A-line STOCK-1, remove 6 standard body-skin pinch grafts, trim off panniculus, and transplant one each to the recipient.

Recipients:

1. M (R) 24 g.
2. M (L) 25 g.
3. M (no) 23 g.
4. M (both) 24 g.
5. M (2R) 23 g.
6. F (no) 20 g.

All ops. RHS and satisfactory.

20 JUN 19 JUN 1953 (11)

Anaesthetise all mice in this group and inspect the grafts together. (Nembutal used) Pass final judgment as to scores only after allowing the grafts to dry in air.

PTO.

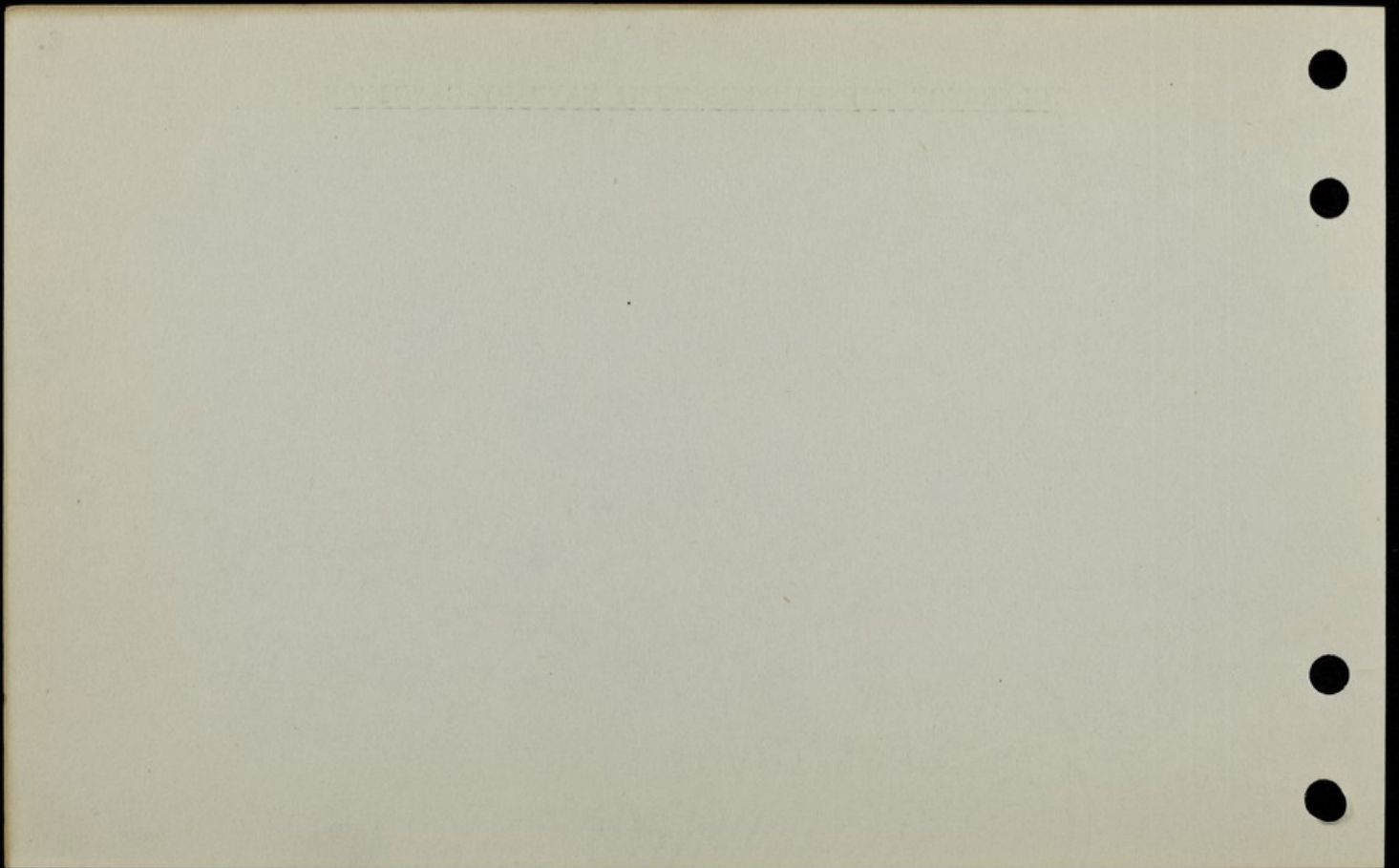
2/6 are clearly without surviving epithelium and have scabbed over completely.

3/6 could conceivably have very slight trace-survival; scabbing is complete except possibly at one or two marginal points.

1/8 can be given a score of about 25% survival; small islets of epithelium are surrounded by large patches of scabbed graft.

This result is in complete agreement with the control series at MST.

TOLERANCE EXPERIMENTS WITH MAMMARY CARCINOMA



This tumour arose spontaneously in A-line female of breeding pair 7A3/P, and was large on 6 Jan 1954. Histology suggested mammary carcinoma.

FIRST PASSAGE: 14 Jan 1954. Excise tumour: note extensive central necrosis. From clean cortical matter prepare standard trochar implants and inoculate into 4 Stock IX A-line females s/i. Growths were more than pea-sized 14 days after grafting. Takes in 4/4.

1 FEB 1954 Excise tumour from 1/4 of the first passage hosts.

(1) Cut up one portion into standard trochar implants and inoculate s/i into 8 CBA mice of litter CX1/5. **EXP TUM-5.**

(2) Prepare a fine tissue suspension as follows. Cut up tumour into small pieces and suck in and out of pipette. With another portion, squeeze gently with glass pestle. Allow the suspended fine matter to sediment naturally, and withdraw the upper layer of the sediment, consisting of single cells and fine organized aggregates passing easily into No.26 needle. Use for Exps **TUM-1,2** (separate cards) and as below:-

TUM-3. Inoculate 8 CBA mice, not more than 24 hr old (probably 16 hr) of litter 7C8/4, each receiving 0.005 suspension under the integument to one side of dorsal midline. Return to mother.

TUM-4. Inoculate 2 A-line Stock IX females bilaterally, subdermally, with 0.005 on each side.

8 FEB 1954 (7)

- TUM-~~3~~ 7/8 CBA's have tiny hard palpable nodules.
TUM-4 Neither A-line mouse has any palpable tumour.
TUM-~~3~~ 0/8 members of the newborn CBA's show any trace of tumour.

6 MAR 1954 (33)

- TUM-3 (New borns) 5/8 = 0. 1/8 has tiny (? regressing) soft diffuse nodule. 2/8 have apparently growing tumours.
TUM-4 1/2 has hard pea-sized ? intracutaneous nodule. 1/2 = 0.
 MAINTAIN TUMOUR THROUGH THIS MOUSE
TUM-5 0/8 growths - evidently regressed. DESTROY.

10 MAR 1954 (37)

- TUM-3 (New-borns) TRANSFER TO SEPERATE SHEET * test-operation.
TUM-4 As noted above, 1/2 has hard peasized tumour, whilst the other mouse is negative. Destroy the latter, and use the small tumour for THIRD PASSAGE: Standard inoculum on RHS of 2 A-line Stock 11 males, and 2 A-line Stock 12 females. In addition - inject subcut. on LHS 0.01 cc of tumour cell suspension.
Also inject 0.05 cc of cell-suspension intraperitoneally into

2 A-line Stock 11 males in attempt to turn tumour into **Ascetis tumpur.** (Picric)

TUM-6 Inject cell-suspension (0.01 cc) into new-borns of 8C4/P (8C4/7) - 6 recips. **See sepearte sheet.**

TUM-7 Control experiment (as for TUM-5): inject standard inoculum on RHS subcut. of 5 mice representing 6th litter from CX1/P, as well as into CX1/P (male and female). Total of 7 mice. **See sepearte sheet.**

18 MAR 1954

TUM-4 **ALL** 4 of the tumour-inoculated A-line mice have no trace of palpable tumour.

30 MAR 1954

TUM-4 S/C series: 3/4 RHS tumours, of which 2/3 have just palpable tumours on LHS (suspension)
1/4 no RHS tumour, but nodule on LHS

I/P series: no abdominal swelling.

19 APR 1954

TUM-4 S/C SERIES:- 3/4 have bilateral ulcerating tumours. **Kill.**
1/4 RHS only. **Keep for propagation.**

I/P SERIES:- 1/2 implantation growth. 1/2 = 0

24 APR 1954 4th passage of tumour.

Kill last 3rd passage survivor, select healthy-looking peripheral region of tumour, and transplant standard grafts to RHS of 3 accredited A-line males.

30 APR 1954 TUM-4. Kill the two A-line mice which had received i/p injection of tumour suspension. No i/p fluid with asstes tumour, but a number of small masses in various parts of body cavity.

Use the large and healthy tumour at impalnatation site of one of these mice for inoculation of newly hatched chicks.

31 MAY 1954 4th passage tumour (cont). See 24 April. All 3 have tumour masses.

1 JUN 1954 FIFTH PASSAGE OF TUMOUR. Excise healthy sections of the tumour on 1/3 mice of the 4th passage. Inoculate in usual way into three A-line Stock XIV males.

NOTE:- Homogeneous inoculum used also for R-EMB 15, q.v.; for TUM-14 q.v.

This tumour was very friable, in the sense that whole tumour on freeing from connective tissue sheaths broke up into small cell clumps. Cells looked perfectly healthy microscopically.

24 JUN 1954 5th passage. Tumour masses in 2/3.

8 JUL 1954 6th passage. Tumour large in 2/3 mice, not at all in 1/3. Transfer in usual way by trochar to 3 A-line stock 18 females.

18 AUG 1954 Use 1 of the 6th passage mice for Tum-17. Reasonable tumours now in 3/3.

21 AUG 1954 From 1/2 remaining 6th passage tumours take healthy cortical matter for

- a) TUM-18 expmt.
- b) 7th passage. 3 stock 20 A-line females. Picric mark. 4-6 standard large trochar implantations lumps.

21 SEP 1954 8th passage. Tumours of 7th passage have not grown very well - they are small, rather hard and with tendency to ulcerate whilst still very small. Transfer in usual way to 3 females.

20 OCT 1954 Tumours: 1/3 no tumour, 1/3 small tumour which ulcerated very soon - mouse had to be killed. 1/3: smallish and rather hard tumour. Use this for

- a) TUM-19 and 20
- b) 9th passage. 3 A-line females.

20 NOV 1954

9th passage not very successful: 1/3 no trace;
2/3 with very small and rather hard nodules.

Something has clearly happened to this tumour over the last few transfers: both its capacity to grow in A-line mice, and its growth-rate, have deteriorated. So has the quality of the tissue itself.

1 FEB 1954 Donor material: As for TUM-3,4 q.v.

Recipient:- Foetuses of female of litter 8C3/2, pregnant by litter mate, first pregnancy. 32g.

See 7 foetuses, age 15 days approx., and inject each so far as possible with 0.005 ml. Visualization and presentation only moderate.

5 FEB 1954 Litter of 6 born overnight - age at time of injection therefore 15-16 days. One or two of the new-borns look rather delicate and may not suckle.

6 FEB 1954 4 left, very undersized & feeble.

8 FEB 1954 3 survivors, and these are still undernourished.

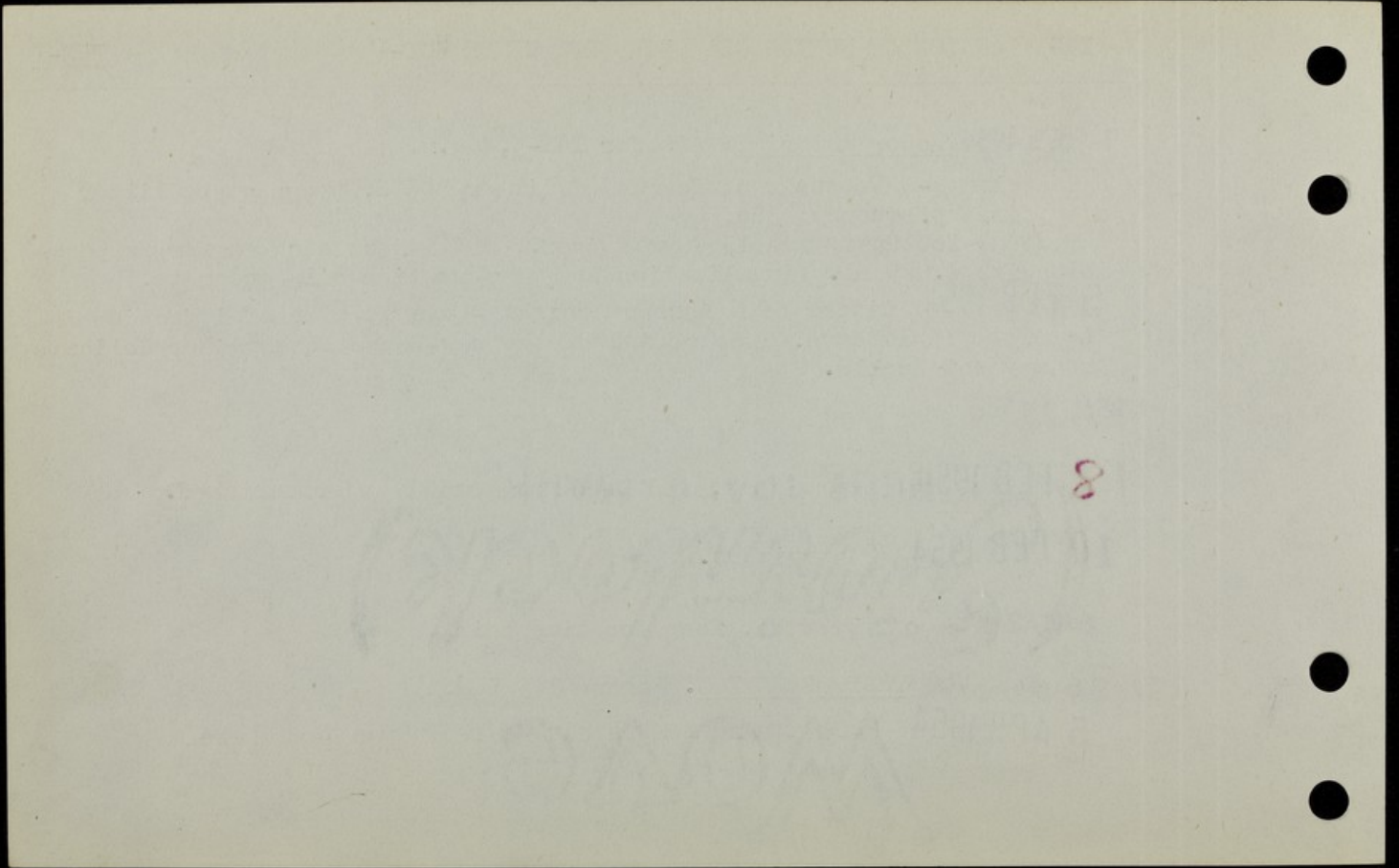
10 FEB 1954 2 survivors.

6 MAR 1954 o/2 growths. Keep for time being.

26 MAR 1954 TEST OPERATION. Donor: 8A5/3 M.

5 APR 1954 (10) Both grafts with breakdown complete.

FEB 1954



1 FEB 1954 Donor material:- As TUM-1.

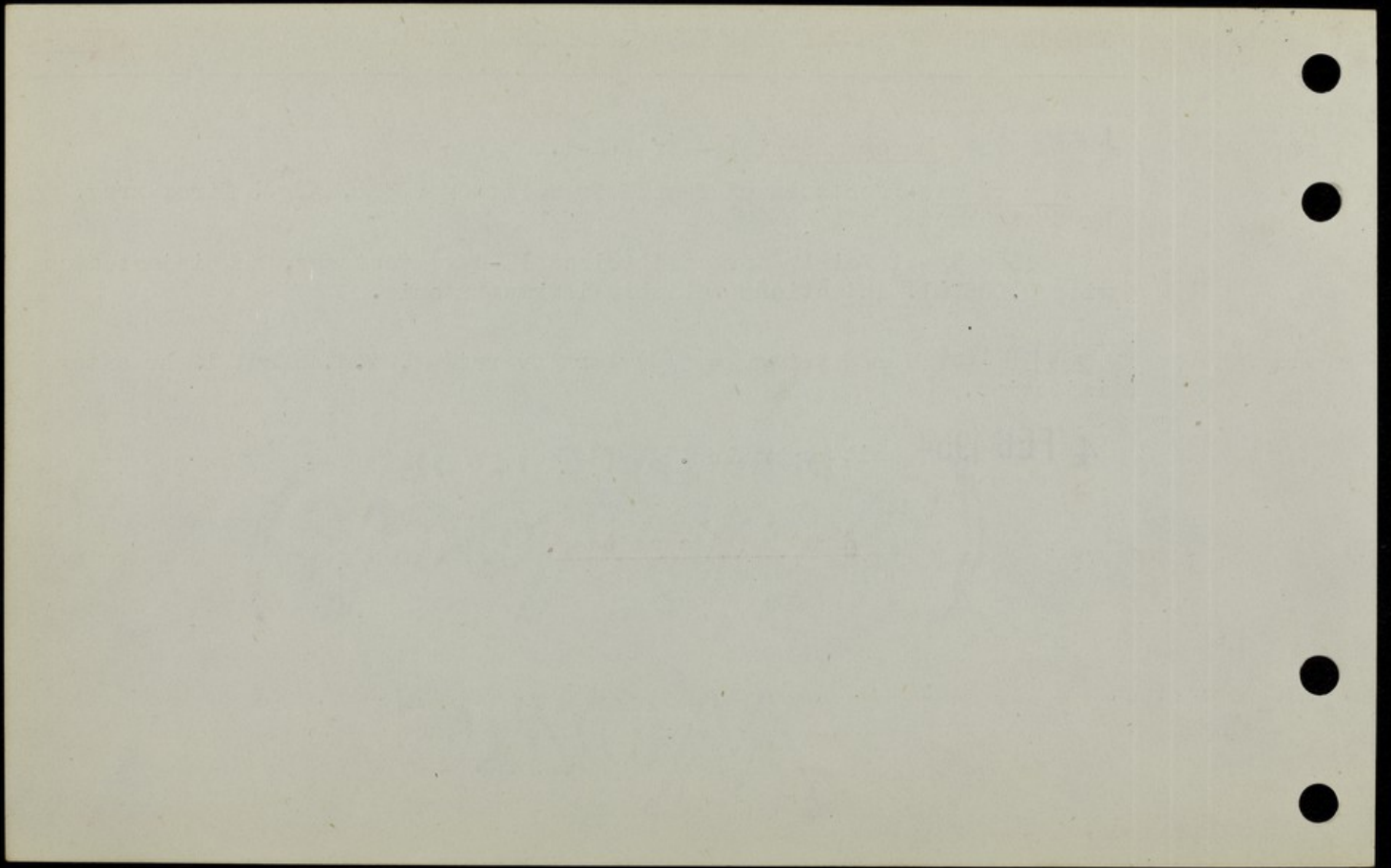
Recipient:- Foetuses of female from litter 6C7/6, 32g., first preg. by litter mate.

See 3 + 1 fairly advanced (circa 18-day) foetuses, and inject each with 0.005 ml. Injections reliably intraembryonic.

2 FEB 1954 Three young (= 3/4) born overnight, i.e. about 12 hr after injection.

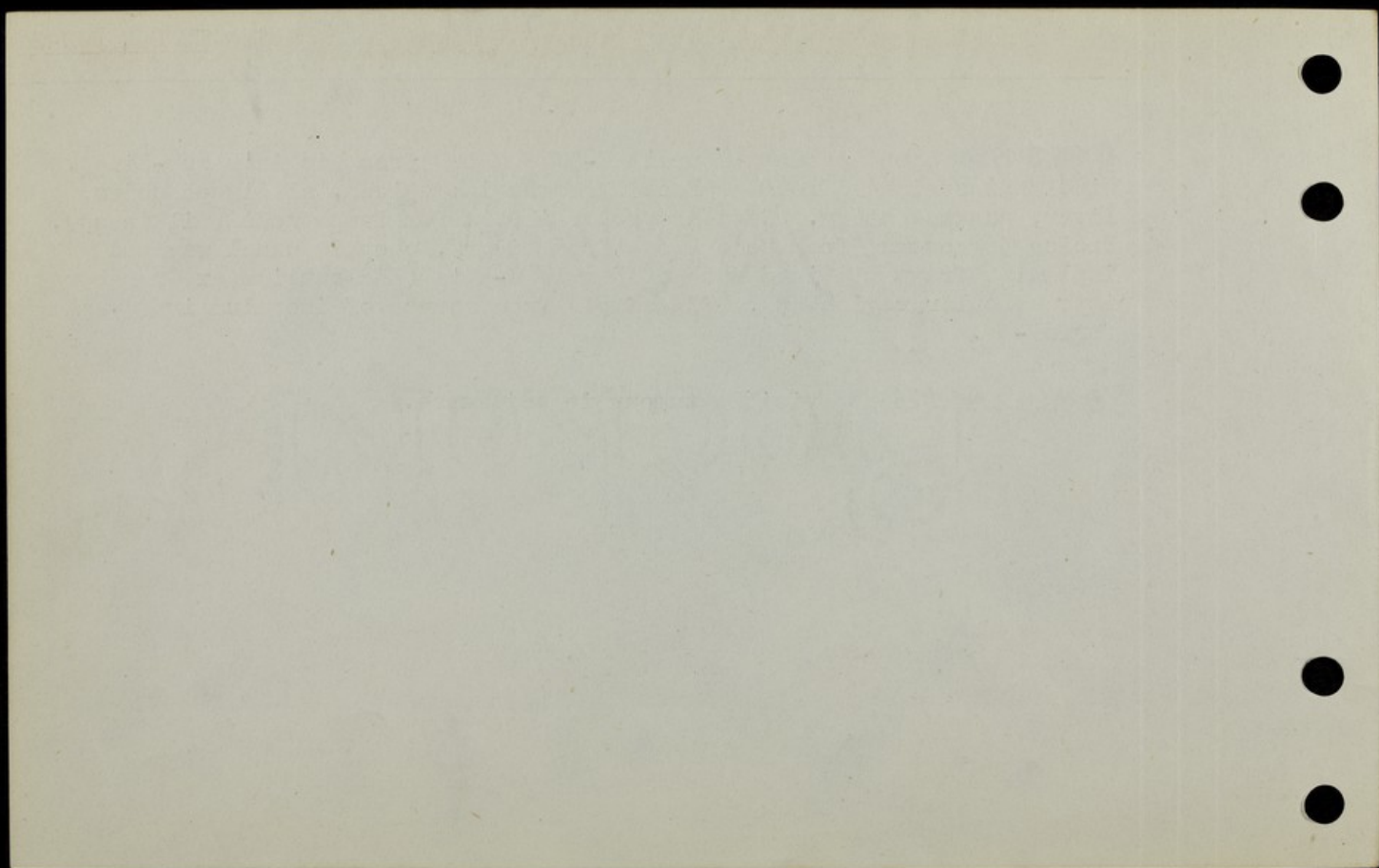
4 FEB 1954 Litter mauled.

Experiment concluded.



8 FEB 1954 Spontaneous tumour in C-57 female (from Michie's sub-line). Widely disseminated rounded focal growths in: spleen, all lymph nodes, liver, gut mesentery. Cutting properties as for lymph node (milkiness). Excise 3 growths (from node & one from liver), chop in usual way and implant 6 fragments into each of 3 male C-57's (**REB** subline ex Gruneberg) into scapular region RHS. **FIX** sample from source of inoculum in formol-Hg.

6 MAR 1954 (26) No **visible** tumour in any mouse.



INOCULATION OF A-LINE MAMMARY CARCINOMA CELL-SUSPENSION
INTO CBA NEW-BORNS.

TUM-3

Donor material: First passage tumour. Prepare cell suspension by cutting up tumour into small pieces and sucking in and out of pipette. With another portion, gently squeeze with glass pestle. Allow the suspended fine matter to sediment naturally, and withdraw the upper layer of sediment, consisting of single cells and fine organised aggregates passing easily into No.26 needle.
Tumour: UCZ-1.1.

Recipients: 8 cba mice, not more than 24 hrs old (probably 16) of litter 7C8/4, each receiving 0.005 cc suspension under the integument to one side of the mid-line.

Date of inoculation: 1 Feb. 1954.

10 MAR 1954 (37) 3/8 mice have large palpable growing tumour. 2/8 have small, hard and rather bony nodules, and 3/8 have no sign of tumour-growth.

Test-operate all mice with A-line skin-grafts.

Donor: A-line Stock 11 male. Standard grafts.

Recipients: 8 mice as listed below:

1. R ear once. No visible sign of tumour. **RHS opn.**
2. L ear once. Large palpable hard subcutaneous tumour on LTW. **RHS opn.**

3. Both ears. Hard palpable (bony) nodule slightly to right of mid-dorsal line in posterior thoracic region. **RHS opn.**
4. No notch. Fairly large subcut. palpable hard tumour slightly to right of mid-dorsal thoracic region. **LHS opn.**
5. R ear twice. Fairly large and diffuse soft and elongated tumour along LHS. **RHS opn.**
6. L ear twice. No visible sign of tumour. **RHS opn.**
7. 2R, 2L. Tiny palpable and slightly mobile nodule (match-head size) almost mid-dorsal in region of chest. **RHS opn.**

Operations generally satisfactory inspite of tumour masses.
Inspect at 8 days - **lok out for immune effect.**

8. Eosin on plaster. No sign of tumour. **RHS opn.**

18 MAR 1954 (8) First Inspection.

1. Healing of test graft perfectly satisfactory but breakdown complete - scabbed over on drying in air.
- 2.* Skin graft 100% but a little puffy & perceptibly pink. Tumour still seems to be flourishing.
3. Very high degree of survival of skin graft. No change in the 'bony nodule' (? tumour).
- 4.* Healing of the graft satisfactory but breakdown complete as evidenced by scabbing on drying in air. Tumour at least 15mm long and seems O.K.

- 5* Healing of graft satisfactory but breakdown far advanced as evidenced by extensive scabbing on exposure. No change in condition of the tumour which is possibly even larger than at grafting operation.
6. Healing of graft O.K. degree of survival about 75% - confirmed by allowing it to dry in air.
7. Healing O.K. but breakdown of graft complete as confirmed by drying in air.
- 8* Graft shows 100% survival but is rather pink. Although no tumour noted at the test operation, there is now a large deep-seated one on LHS.

Comments: All animals now bearing definite and apparently surviving palpable tumours - 4/8 - are marked * above. Healing of all the grafts entirely satisfactory and in no way prejudiced by the presence of a large tumour mass. REB & LB suspect that 'immune' b.d. took place in some of the test grafts viz. 1, 4 & 7.

19 MAR 1954 /9/

- 1, 2, 4, and 7 : breakdown complete.
3. About 25% survival.
5. Mere trace survival.
6. About 50% survival - half of graft has scabbed.
8. 100% survival, but deep-seated pinkness and slighty puffy.

20 MAR 1954

(10) The only grafts with any survival are the following:

2. Now less than 25% survival.
3. Mere trace survival.
8. Still 100% survival - no contracture. This graft may well show marked prolongation.

21 MAR 1954

(11) MST

2. Breakdown complete.
3. Trace survival.
8. Complete survival - graft has lost its reddening, and though still not autograft-like is now looking healthier.

24 MAR 1954 (14).

1. No tumour palpable.
2. Tumour now very large- growth obviously continues 25mm x 20mm.
3. The previously reported 'bony nodule' has grown enormously and is now at least 10mm long.
4. Tumour firm but hasn't grown conspicuously since last inspection. measurements - 15mm x 7mm.
5. There are two separate tumours close together on LHS. The larger one is the more ventral of the two, is disc shaped- diameter about 14 mm. Has definitely grown.
6. No tumours palpable.
7.

8. Skin graft 100%. The improvement has continued but there is no hairgrowth and surface is slightly shiny. Tumour has grown, now 15mm x 15 mm.

30 MAR 1954 (20)

8. Mouse died over week-end 27/29, found dead on 29th. Graft excised - appears O.K. but no sign of hair-growth. Specimen TUM-3.8. Formpl meerc.
Open body cavity: large growing tumour attached to perineum.
~~to be seen~~
2. Tumour 3 cm diameter - obviously viable and growing. Kill mouse.
3. Tumour mass on dorsum of thorax, firmly attached to spine. 14 mm long - clearly growing.
4. Tumour has not grown since last inspection and may be regressing.
5. Tumour pair have coalesced, and clearly grown. (now less flat and almost rounded). 20 mm long.

1, 6, and 7. No palpable tumour present. Kill.

11 APR 1954 Kill 3 and 5, leaving 4 alive. Use tumours in experiments TUM-3.A and B.

3. Large healthy-looking tumour closely attached to spine. Well vascularised; rather pulpy when cut up.
5. Several large tumours on LHS. One is particularly large and may be partly necrosed. The smaller ones look very healthy. Use these.

Take biopsies from both and fix in Formol/Hg.:

Specimens TUM-3.3 and 5.

19 APR 1954

4. Tumour has now regressed completely: KILL.

INOCULATION OF A-LINE MAMMARY CARCINOMA UCZ-1
INTO A-LINE ADULTS AFTER RESIDENCE IN CBA HOSTS

TUM-3.A

1 APR 1954

Remove tumours from TUM-3.3 and 5. These have now been in residence in cba hosts for 8 weeks.

Cut up seperately into tissue culture fragments, and implant standard inoculum on RHS thorax of the following recipis: 3 and 3 Stock 12 A-line males.

3 mice - R NOTCH Tumour from TUM3.3.

3 mice - NO NOTCH " " TUM-3.5.

Implantations subcut. Standard trochar. Mice lightly anaesthetised.

15 APR 1954 (15) 1 no notch mouse has tumour about 2-3 mm diameters, and 1 R notch mouse with tumour nodule. The rest appear to be negative.

REFERS TO 3B

19 APR 1954 (18)

SERIES 3.A (~~C~~^A-line recipients).

NO NOTCH GROUP:- 3/3 small hard nodules.

R NOTCH GROUP:- 3/3 = 0

1 MAY 1954 (30). There are firm palpable tumour masses in all 6 recipients. Keep.

INOCULATION OF A-LINE MAMMARY CARCINOMA UCZ-1
INTO CBA ADULTS AFTER RESIDENCE IN CBA MICE

TUM-3.B

11 APR 1954

Exactly as for TUM-3.A., but here im-
plant tumours into CBA adult mice.

Recipients: 5 ex-breeding pair females (7C6/P, 7C4/P, 7C5/P,
7C3/P, 8C1/P) and 1 stock 15 female.

3 mice - R NOTCH Tumour from TUM-3.3

3 mice - NO NOTCH " " TUM-3.5.

14 APR 1954 (13) 3/6 show no trace of tumour - all
these are R notch mice.
3/6 (all no notchers) have very small
tumour nodules.

REFERS TO 3A

1 MAY 1954 (30). No tumour palpable in any of the 6 mice
inoculated. KILL them.

19 APR 1954 (18) 0/6 tumour nodules. Thus either no growth or, in
two caees, regression.

AC 51 25

INOCULATION OF A-LINE MAMMARY CARCINOMA CELL-SUSPENSION
into CBA NEW-BORNS.

TUM-6.

10 MAR 1954

Donor material: TUM UCZ-1.2. (2nd passage). Prepare cell-suspension - the tumour disintegrated very easily on cutting up. It was a intra-dermal, about the size of a large pea. The suspension thus produced contained numerous cell-clumps easily visible to the naked eye.

Recipients: 6 new-born mice (representing litter 8C4/7) about 8 hours old - certainly not more than 12 hours. Inject 0.01 cc in mid-dorsal line, sub-integumentary. Injections were with syringe vertical, so that many cell-clumps were injected.

11 APR 1954 (21) 3/6 with palpable tumour on dorsum of thorax.

5th 12 APR 1954 (25) 3/6 with palpable tumour, about 1cm in diameter.

14 APR 1954 Test-operation with A-line skin. (34)

3/6 mice with large tumours in region of neck. Operate all 6 with A-line skin grafts on RHS. Donor: Stock 12 A-line male.

~~15 APR 1954~~

21 APR 1954 (7) Mice without tumour: grafts have healed satisfactorily. Breakdown is far advanced - grafts largely scabbed shortly after exposure. But still slight survival. Could be a weakish immune effect.

Mice with tumour: All 3 tumours have grown in size since transplantation of grafts - impression is that tumours are surviving. They are now very large.
Notch mice:

(R) Cuticle very adherent. Advanced breakdown, but certainly still some surviving epithelium.

(D) Cuticle peels easily. Graft 100% surviving, but epithelium does not look too robust; slight haemorrhagic spots. This mouse has largest tumour of all.

(no) Slight dorsal overlap, but not serious. Cuticle very adherent; exceedingly difficult to remove. Graft almost completely scabbed some after exposure. Breakdown probably complete. Definitely immune effect.

22 APR 1954 (8) 2 mice without tumour: breakdown complete.
Score: 7 - 8 days.

(R) 100% survival, but graft does not look healthy & prognosis poor.

(L) Still some survival.

(no) Breakdown complete. Score: 7 days.

23 APR 1954 (9)

(R) Still high degree of survival, but breakdown has certainly begun.

(L) Still some, but now rather slight, survival.

26 APR 1954 (12)

(R) High degree of survival - scabbing only marginal.

(L) Still some survival in centre.

27 APR 1954 (13)

(R) Still some survival, but scabbing is extending and graft now becoming discoloured.

(L) Slight survival.

1 MAY 1954 (16)

Kill ~~both~~ mice without tumour.

(R) Tumour enormous, 6.2 g. and 3cm x 3 cm. Has clearly grown since transplantation of ~~graft~~ skin graft.

Skin graft: extensively scabbed, but between scabs there may still be islets of survival.

Kill mouse. Tumour specimen: TUM-6.R. Pretty healthy

Graft specimen:

(L) Tumour mass large, 3.2 g., $2\frac{1}{2}$ x $2\frac{1}{2}$ cm - has clearly grown since skin grafting. Tumour pretty healthy.

Skin graft: appears to have overcome the reaction, the scabs have cleared and there appears to be surviving epithelium. But overgrowth cannot be entirely ruled out.

Kill mouse.

Tumour specimen: TUM-6.L.

Graft specimen: "

(no) Tumour considerably smaller than in the others and ~~see~~ has not grown so appreciably since skin transplantation; impression is that it may be suffering from reaction.

Kill mouse. Tumour specimen: TUM-6.no.

Tumour with some necrosis, but also some healthy tissue. Select healthy part for biopsy.

INOCULATION OF CBA ADULT & MICE WITH A-LINE MAMMARY CARCINOMA -
CONTROL EXPMT.

TUM-7

10 MAR 1954 Donor material: TUM UCZ-1.1 (2nd passage). Standard tumour inoculum.

Recipients: Mice of 6th litter from CX1/P, b. 23 Jan. - 5 mice. Standard RHS inoculum.

Also CX1/P (male and female) themselves.

Total number of mice: 7.

18 MAR 1954 Hard pin-point nodule palpable in every mouse - presumably this is the regressing tumour implant.

30 MAR 1954 (20) 5/7 have no trace of tumour.
2/7 have the slightest remnant of nodule.

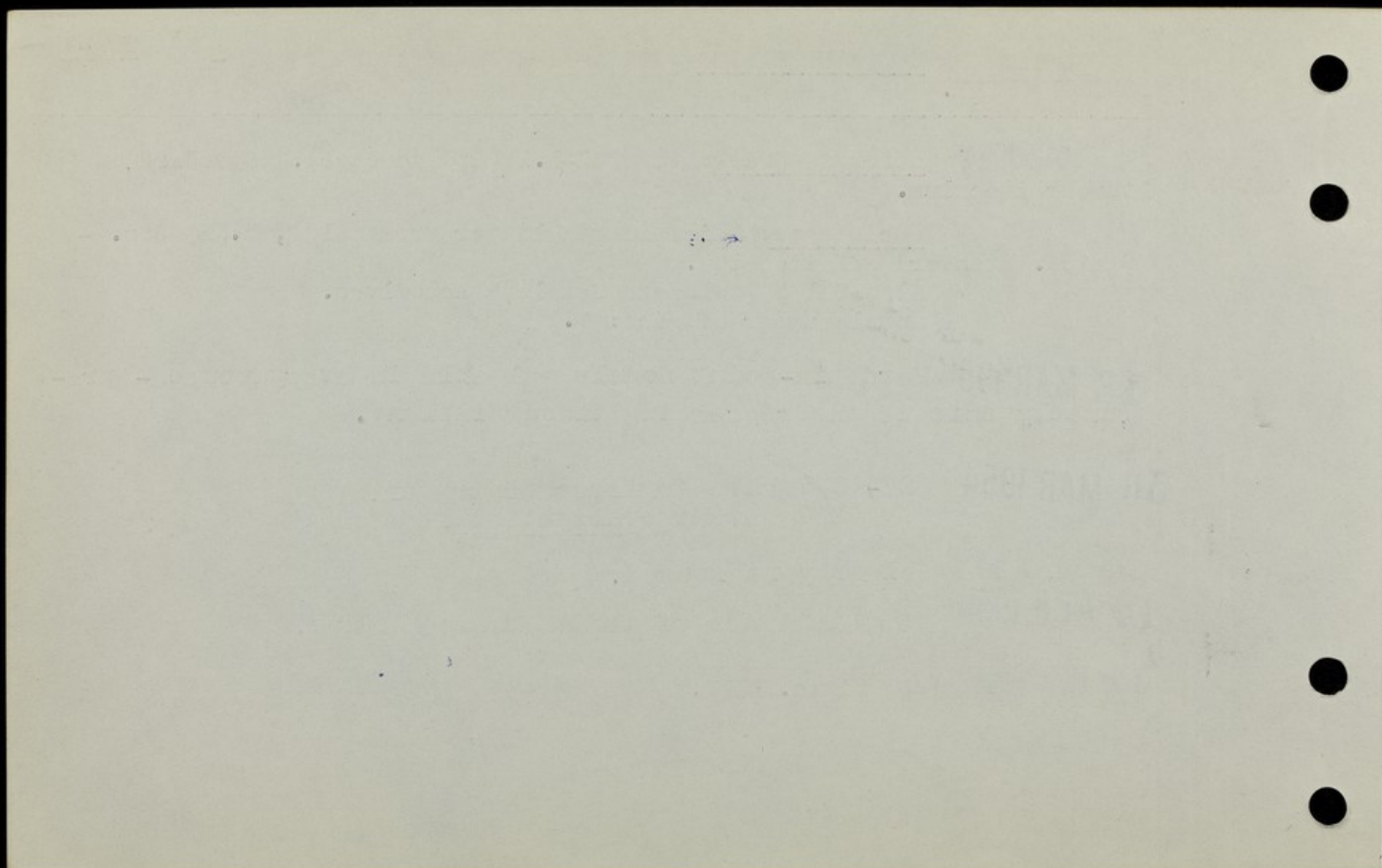
Tumours have clearly regressed.

14 APR 1954 (35) No trace of tumour in any mouse.

19 APR 1954 (40) Ditto. KILL.

EXP CONCLUDED

18 MAR 1954



IMPLANTATION OF HETEROLOGOUS WALKER RAT SAR-
COMA INTO CBA NEW-BORN MICE

TUM-8

6 APR 1954 Walker rat sarcoma obtained from Chester-Beatty Institute, and has been growing on this rat for 7 days.

Remove thin slices from peripheral portion of tumour which looks somewhat diffuse and fatty. Cut up into very small fragments with cataract knives - no tendency for tumour to fragment easily, or for cells to milk out. Also prepare a number of normal tumour implants.

Recipients. Litter 808/3, born during the night but later than 10 p.m. last evening. Age at time of injection therefore not more than 12 hours, and probably rather less.

Inject each of the 5 new-borns, in the mid-dorsal line, subintegumentary, mid-way between the scapulae, with 4 small fragments of the tumour. Use smallest trochar. Implantation very satisfactory.

Also carry out the following implantation:

Into each of 2 rats inject LHS with small (new-born) fragments (CONTROL), and RHS with larger standard tumour fragments (2 mm cubes).

13 APR 1954 (7)

This litter is very backward and two of the mice died 2-3 days after birth. Of the 5 survivors, two more died last night - they were emaciated and very stiff when still alive. Kill the rest, which are in not quite so bad a condition but also somewhat pathological.

Note that all 5 mice had a scurfy skin along the back, were rather stiff and immobile, and generally very backward.

Autopsy reveals that both mice which had died naturally had diffuse tumour masses spreading from the dorsal region of the anterior thorax which were probably viable at time of death.

Of the remaining three, 1 had viable tumour mass, whilst the other two showed evidence of tumour proliferation after implantation but probably subsequent necrosis.

Conclusions: Result rather unsatisfactory because tumour was not allowed to run for quite long enough, but there is evidence that (a) the tumour has proliferated considerably in the cba hosts, (b) that tumour is not likely to have been the direct cause of death, and (c) that death may have been due to passenger pathogens or toxicity.

INOCULATION OF HETEROLOGOUS WALKER RAT SARCOMA TUM-9
INTO A-LINE NEW-BORN MICE

6 APR 1954

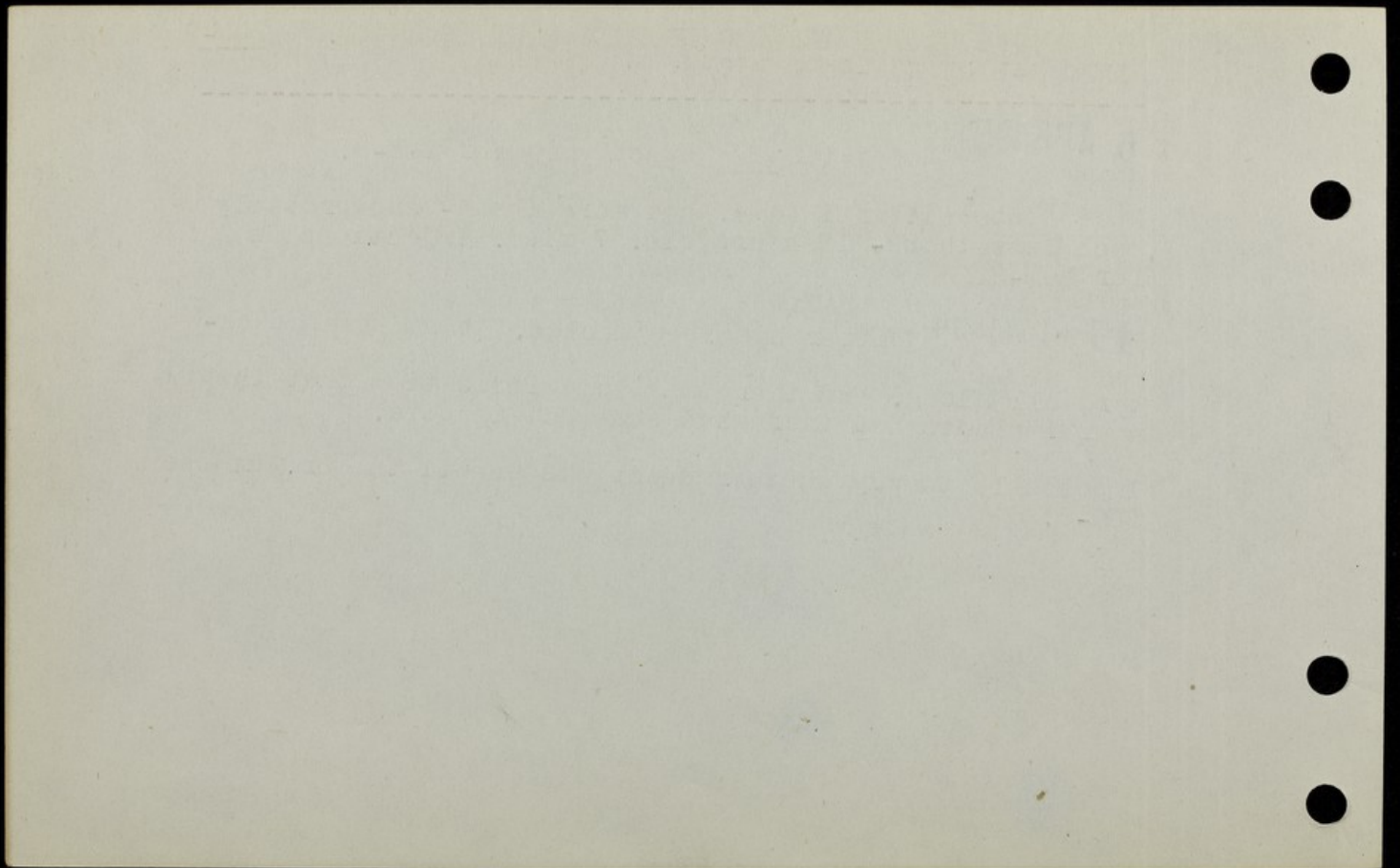
Donor tissue: Exactly as for TUM-8.

Recipeints: Litter 10A4/4, not more than - and probably much less than - 12 hours old, 7 mice. Injections as for TUM-8.

11 APR 1954

Litter has been mauled. It had been backward all along, and there was some indication that the tumour implants had increased somewhat in size.

Impossible to say whether deadt was due ti tumour. But see TUM-8.



INOCULATION OF UCZ-1 A)LINE MAMMARY CARCINOMA
CELL SUSPENSION INTO CBA NEW)BORNS

TUM 10

24 APR 1954

-- Recipients: 10C5/2.1-7, less than 24 hours, and probably 16-20 hrs. old. Inject subcut., in mid-dorsal region, 0.02 ml of cell suspension.

Donor: A-line carcinoma UCZ-1 removed from 3rd passage mouse. Cell suspension rather fine. Note relative great age of new-borns.

31 MAY 1954 (37) 6/7 = 0; 1/7 shows small RHS nodule. NOTE FINE SUSPENSION USED: cf. exp. TUM- 12. Keep.

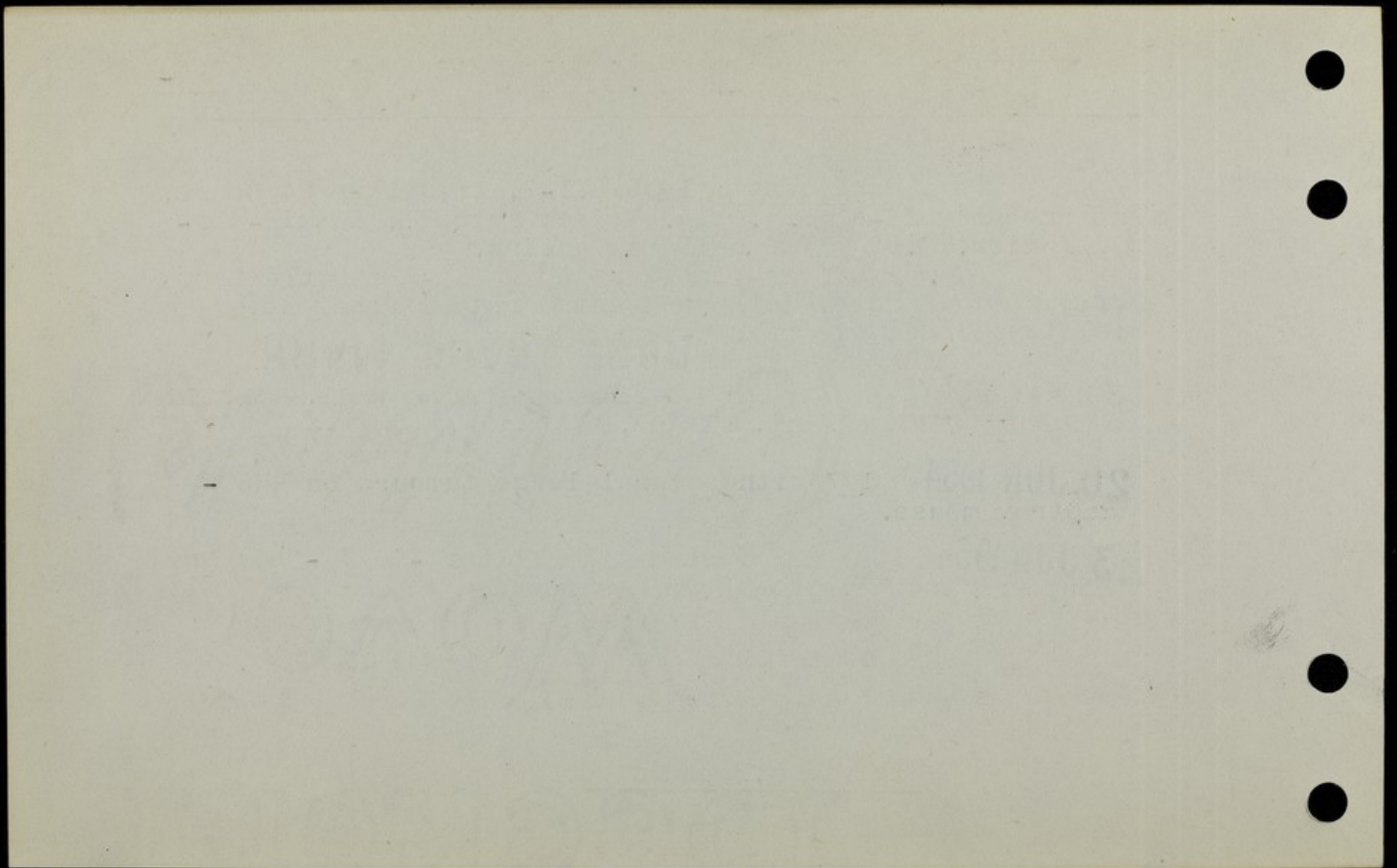
20 JUN 1954 1/7 with several large tumours on RHS - destroy mouse.

23 JUN 1954 (60) The rest of the litter - 6/7 - have no tumours and have now been destroyed.
Note age of mice when injected, and nature of suspension.

20 JUN 1954 1/7 developed tumour masses.

Experiment concluded.

20 JUN 1954



INOCULATION OF UCZ-1 A-LINE MAMMARY CARCINOMA
CELL SUSPENSION INTO C57 NEWBORNS

TUM-11

24 APR 1954

Donor: As for Tum-10.

Recipients: C57 litter, about 36 hours old. 7 mice. Inject into each 0.02 ml subcut. in mid-dorsal region. Note age of mice.

30 APR 1954

Mice O.K.

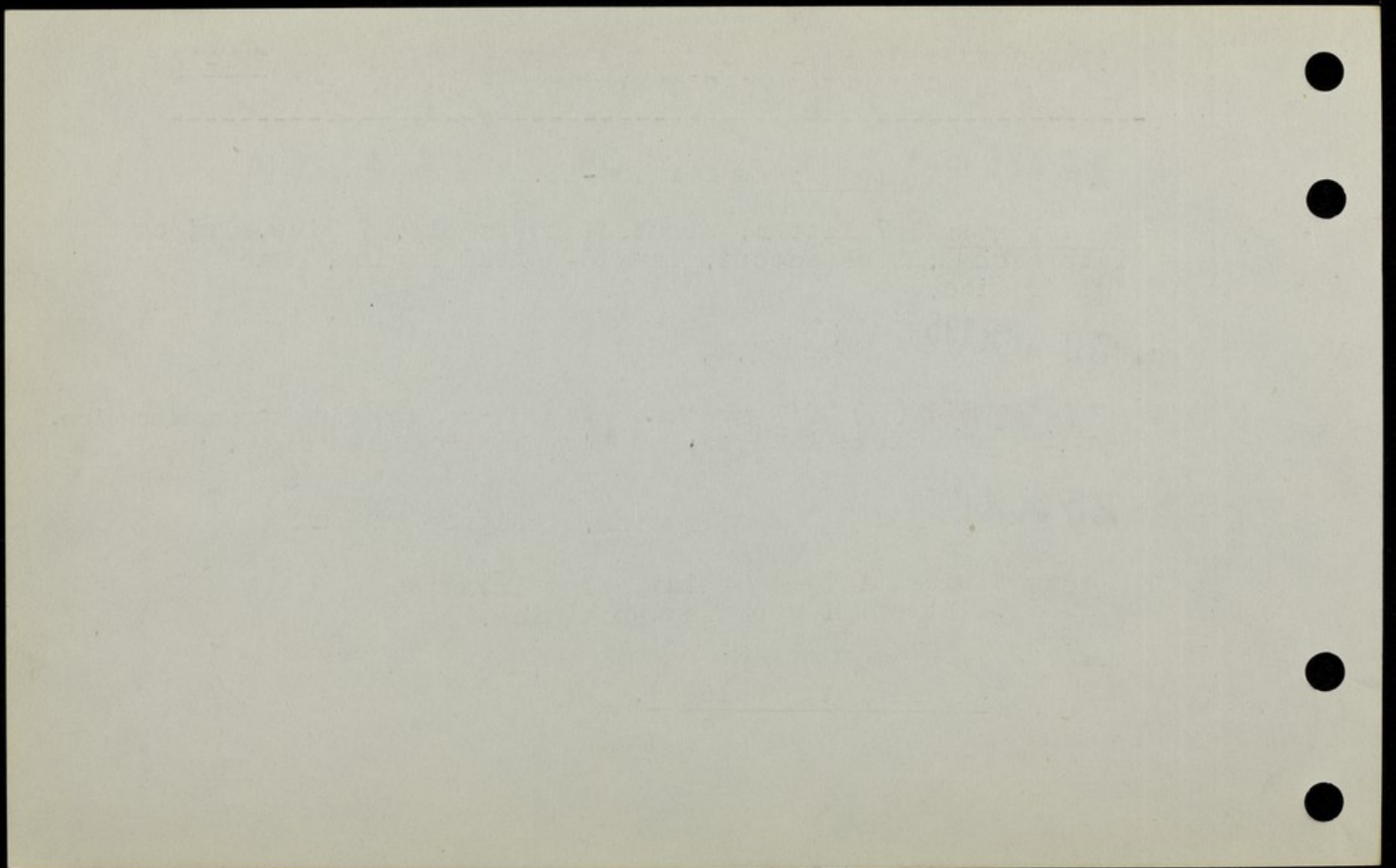
31 MAY 1954 (37) 0/7 growths. Note (a) age, (b) fine tumour inoculum. Nothing much can be inferred; but keep for a few more weeks.

23 JUN 1954

(60) 0/7 growths. Destroy litter.

Age of mice at time of injection invalidates this expmt. from point of view of new-born mice.

Experiment concluded.



INOCULATION OF UCZ-1 A-LINE MAMMARY CARCINOM
CELL SUSPENSION INTO CBA NEW-BORN MICE.

TUM-12

30 APR 1954. Donor: The third passage A-line mouse which was given an intraperitoneal injection of tumour suspension in an attempt to produce ascetes tumour. Mass had formed subcut. at point of inoculation.

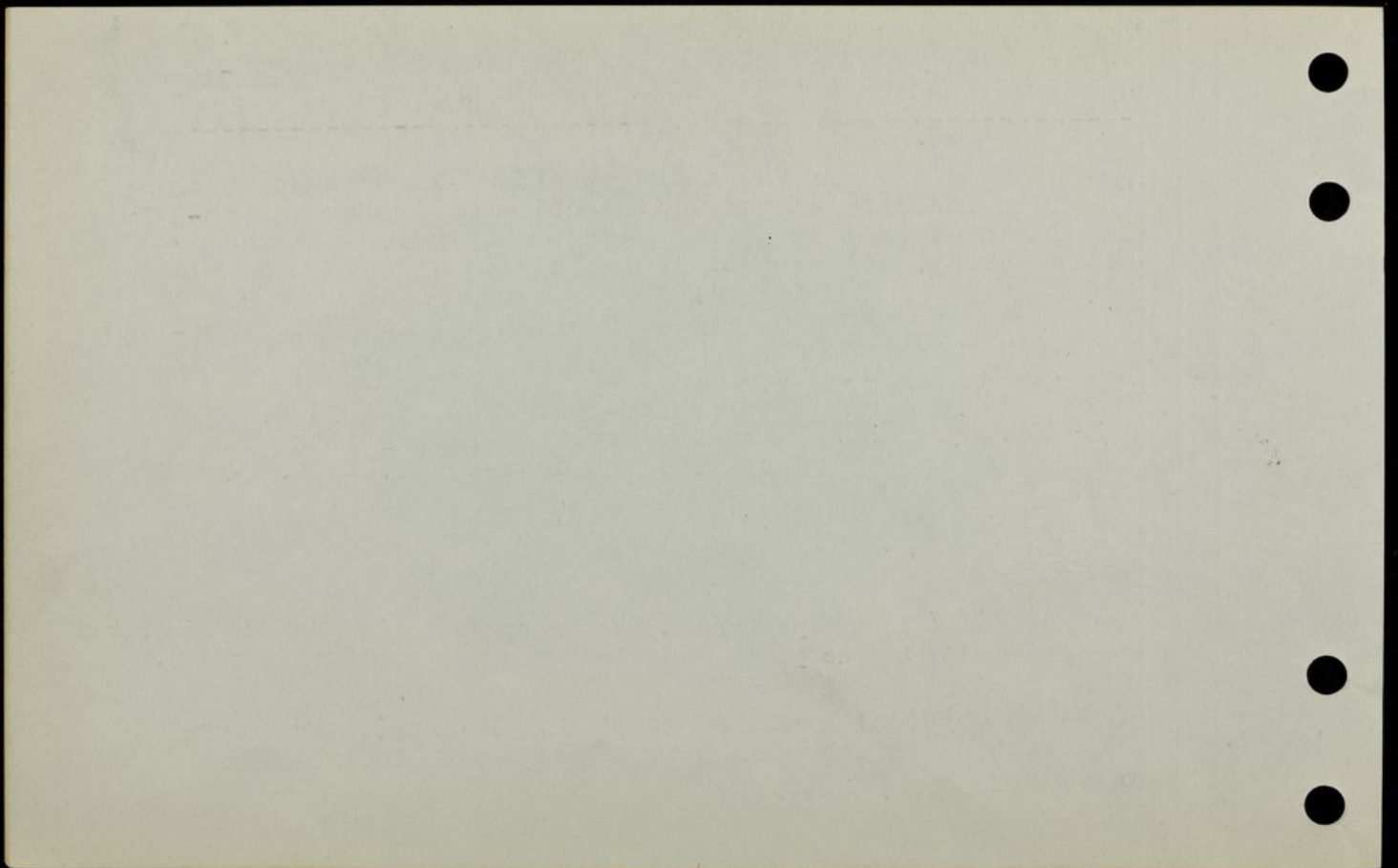
Prepare suspension which was probably somewhat finer than usual.

Recipients: 6 new-born mice of 1002/2.
Age: Not more than 15 hours, probably rather less.
Inject 0.01 and in a few 0.02 ml. Some loss.

~~10 MAY 1954~~

31 MAY 1954 (31) 0/6 positives. But as suspension was unduly fine, can these results be accepted? (Note long delay in earlier controls).

24 JUN 1954 (55) 0/6 have growths. Destroy. Note that the fine suspension used is likely to make this result unacceptable.



INOCULATION OF A-LINE MAMMARY CARCINOMA INTO TUM-13
CBA ADULT MICE

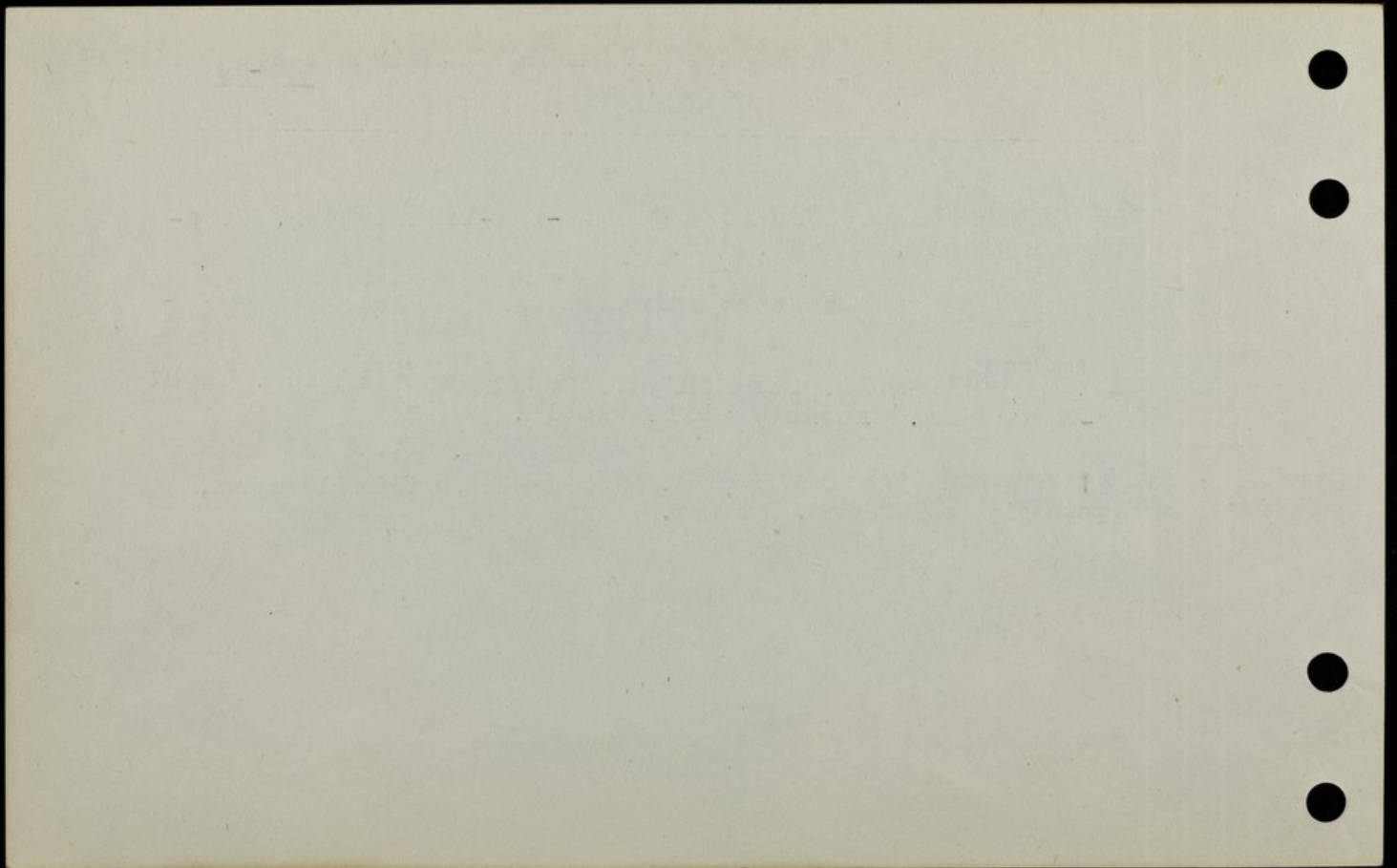
24 APR 1954 Use 3rd passage UC 211 A-line tumour. Standard implants, subcut. RHS.

Recipients: 5 Stock 14 cba males (adult).

1 MAY 1954 Small, firm tumour nodules in all mice, about 2-3 mm diam. Probably still viable.

30 MAY 1954 (37) 0/5 growths. As these had originally grown, the point is established. Destroy.

EXPERIMENT CONCLUDED



1 JUN 1954 Inoculum:- Fine friable cell clumps from 4th passage: see master card for UCZ-1 this day.

Recipients:- Litter 10C5/3 (8 mice), not more than 15 hrs old. 0.01 ml each of the tumour clump suspension, somewhat to one side of mid-dorsal line anteriorly, sub-integumentary. AGLA syringe with wider needle.

10 JUN 1954(9) No palpable growths in any mouse.

24 JUN 1954 ((23) 1/8 with tumour mass (small). 7/8 have no palpable growths.

9 JUL 1954 (38) 1/8 with 5 mm tumour in mid-dorsal line; also smaller mass in lumbar region.
 1/8 with small 2-3 mm tumour - mobile in scapular region.
 6/8 without visible tumour.

17 JUL 1954 (46) Test-operation.
 Graft the mouse with growing tumour in mid-dorsal line.
Donor: Stock 17 A-line female.

23 JUL 1954 (6) Inspect grafted mouse. Graft has healed soundly and shows no sign of immune effect. Rebandage.

28 JUL 1954 (11) ~~Both grafts are~~ completely surviving. Tumours appear to be continuing growth.

31 JUL 1954

(14) ~~Both grafts are~~ almost completely surviving, but there is some contracture and a little superficial scabbing. Impression is that very mild reaction is in progress.

31 JUL 1954

(14) Graft looks healthy if a little delicate. Small patch of incipient hairgrowth.

3 AUG 1954 (17) Enormous tumour in interscapular region, another smaller one in lumbar region attached to spine. Graft is in excellent condition, and is growing a dense crop of albino hairs. SUPER.

6 AUG 1954 (20) Further growth of both tumours. , Graft now bears dense crop of white hairs but it is slightly scabbed & scurfy ventrally.

Tum-14.

9 AUG 1954 (23) Graft 100% with good hairs.

13 AUG 1954 { (73 days since tumour inoc.)
(27 days since skin test graft)

Kill the 7/8 mice in which there is now no sign of a tumour. This gives a score of:

1/8 complete tolerance 1/8 weak tolerance 6/8 no tolerance

Tumour-mouse wt. 31 g, 2 tumour masses wt. 9g, ~~male~~. Excise both carefully through 2 separate incisions, haemostat-cautery method checking bleeding. Very feeble: will die. Give $\frac{1}{2}$ ml saline i/p. E

Transplant excised tumour as under TUM-16A, q.v.

GRAFT of skin has well defined if rather wispy hair-growth with clear margin. Unquestionably 100% survival with hair growth at 27 days. No specimen taken.

24 AUG 1954 (38) Mouse has recovered reasonably well and gained some weight. Graft has 100% survival, and thinnish crop of albino hairs is covering whole graft.

26 AUG 1954 (40) Graft O.K. New tumour has sprung up anterior
RHS thorax, and is now roughly the size of a large pea. Hence
attempt to 'cure' mouse of tumour (as well of graft) by
adoptive transfer of immunity.

*Immune' node donors: 4 ex-EMB-231 mice, with grafts at MST.
Excise the regional lymph nodes - rather small and flabby. Cut
up the 8 nodes with fine cataract knives until small enough to
pass through very coarse syringe needle. Resulting inoculum
is mixture of lymphocyte suspension and small fragments.
Inject i/p. Clip graft.

21
30 AUG 1954 (40 ± 4) Tumour appears to have continued its growth.
So far no detectable sign of graft deterioration.

31 AUG 1954 (40 ± 5) Ditto.

1 2 SEP 1954 (40 ± 7) Graft appears to be unchanged. But note that
tumour growth seems to have discontinued - it may even be regress-
ing.

3 SEP 1954 (40 + 8) REPEAT adoptive immunization. Inject expressed fluid
(sieve method) from 10 immune nodes suspended in total of 1.5 ml normal
citrate saline. Nodes are homolateral axillaries from AD.222-238 series A
q.v., A to CBA, 10 days, mice received $\frac{1}{2}$ mg/day DOC-glucoside.
Graft may have a barely perceptible pinkness.

7 SEP 1954 (40 + 8 + 4) Graft is still in good shape.

The tumour has undoubtedly grown and is almost certainly viable. Note that the overlying skin is considerably flushed - possibly due to reaction?

Mouse has had healthy litter of 7 - a measure of its recovery from the original tumour and the excising operation.

11 SEP 1954 (46 + 40 + 8 + 8). The original RHS anterior tumour is now growing rapidly. A 'new' tumour has sprung up mid-dorsally to LHS, & this is involved with the body wall tissue. Surgical excision clearly hopeless, and two passive immunizations have not stopped growth of tumour or affected the skin graft, which has grown with the host & looks perfectly normal. There may have been temporary inhibition (above).

Excise the new growth and test for acceptability to normal adult CBA mice by bilateral trochar implantations of healthy tissue. (This new growth has sprung up in face of some immunological opposition, & may have changed its characters.) Three mice.

<u>Summary of history:</u>	Day 0 (birth)	Implant UCZ-1
	46	A-line skin graft
	46 + 27	Excise 9g tumour
	46 + 40	First PT trial
	46 + 48	Second ditto
	102 = 46 + 56	Kill: transfer tumor as above

19 OCT 1954 Control mice (normal cba's).

No trace of tumour. Specificity of A-line tumour has clearly remained unaltered inspite of long residence on tolerant host. Kill.

Experiment concluded.

3 JUN 1954 Inoculum: 4th passage tumour (not same as in Tum-14). Tumour material very healthy; friable, & passed easily into wider bore Agla needle.

Recipients:- Litter 7C9/7, not more than 16 hrs old (one mouse mouse only had been born 6 pm last night: inoculations at 11.30 a.m. this day.

Give each 0.01 ml suspension to right side of dorsal midline below integument, & another 0.005 in same way on LHS.

24 JUN 1954 (21) 1/6 has palpable tumour mass. The rest are so far negative.

9 JUL 1954 (39) 1/8 with very large tumour RHS.
 1/8 with 2 small masses, about 3 mm plus, on dorsal surface of thorax.
 1/6 with single 5 mm tumour on dorsal side of thorax.
 3/6 have no visible sign of tumour.

17 JUL 1954 (47) Kill mouse with enormous tumour - almost dying from it.

Test-operate the other 2 tumour-bearing

mice - tumour clearly growing. Donor: Stock 17 A-line female.
Standard grafts and ops.

23 JUL 1954 (6) Inspect the 2 grafted mice. Healing sound.
Some haemorrhagic patches, but epithelium firm and no sign
of immune effect. Rebandage.

28 JUL 1954 (11) Complete survival of grafts. Leave unbandaged.
Slight puffiness; scaly cuticle.

31 JUL 1954 (12) Complete survival: grafts look healthy, if some-
what delicate, and has small patch of incipient hair-growth.

31 JUL 1954 (14) Both grafts are almost completely surviving, but
there has been some contracture and a little superficial scabbing.
Impression is that very mild reaction is in progress.

3 AUG 1954 (17) Number the 2 mice as follows:

1. (R) Large tumour (growing) on left scapula. Skin graft un-
contracted, 100% survival, but with smooth and scurfy cuticle;
no hair-growth.
2. (no) 2 growing tumours - one in interscapular region, the other
over l. kidney. Skin graft with some contracture; slight ven-
tral scab, roof smooth and shiny; no hair-growth except for
a few isolated hairs in centre.

6 AUG 1954 (20).

1. Graft 100%, uncontracted but slightly rather smooth & shiny and completely bald.
2. Graft somewhat contracted with smooth shiny surface & only a small tuft of rather wispy white hairs ventrally.

9 AUG 1954 (23).

1. Graft 100%, but tiny dorsal plaques & no hairs.
2. 100% with a few more hairs than hitherto.

13 AUG 1954 (71 days since tumour inoculation)
(27 days since test grafting with skin)

AUG 10⁵ ~~ear~~ ^{son}
TUM-15.1 (ear). M./^{29g} Graft well-defined & firmly epithelialized, but almost bald except for little clusters of very stunted white hairs. Nevertheless 100% survival.

Tumour is a single well-defined mass of $4\frac{1}{2}$ g. Excise surgically by cautery-mosquito-forceps technique; sew up skin after making new edges. Follow the graft. Attempt to 'cure' immunologically if & when tumour grows again.

Fate of tumour: TRANSPLANT AS UNDER TUM-16B q.v.

TUM-15.2 (No mrk). F. Graft well defined, with good dorsal hair growth,

but some scaliness ventrally (as if dried serous matter, but very like early stages of regression produced by e.g. immune node implants). Probably on way out but still surviving.

The tumour in form of two huge masses with spread outward into skin. Make 2 separate incisions & excise surgically, using cautery & haemostats. Tumour weighed 10g! (mouse 30g). DIED UNDER OPERATION, possibly immediate cause of death being haemorrhage.

Fate of tumour: TRANSPLANT AS UNDER TUM-16C

- - - - -

Kill 3 mice which show no signs of present tumour. FINAL SCORE:-

3/6 completely or very highly tolerant (this includes 1 mouse killed at 47th day, non-reoperable).

3/6 no growth.

24 AUG 1954 (38) Mouse has recovered very well. Graft has 100% survival; wispy hair-growth in dorsal half.

13 SEP 1954 (59) Graft much unchanged - not perfect, but 100% survival, wispy hairs. Note that tumour has disappeared completely.

P.T.O.

TUM-15

13 SEP 1954 (59) REOPERATE on LHS with large A-line
(stock 22 F) skin graft.

24 SEP 1954 (59 ± 11) RHS (old): graft surviving.
LHS (new): most of graft scabs af-
ter drying - about 25% survival dorsally.
A strange result.

25 SEP 1954 (59+12)
Old Graft - now bears scab covering about 1/3 of its area
and suggests that this graft is going
New Graft - completely scabbed except for small epithelialised
and surviving portion dorsally.

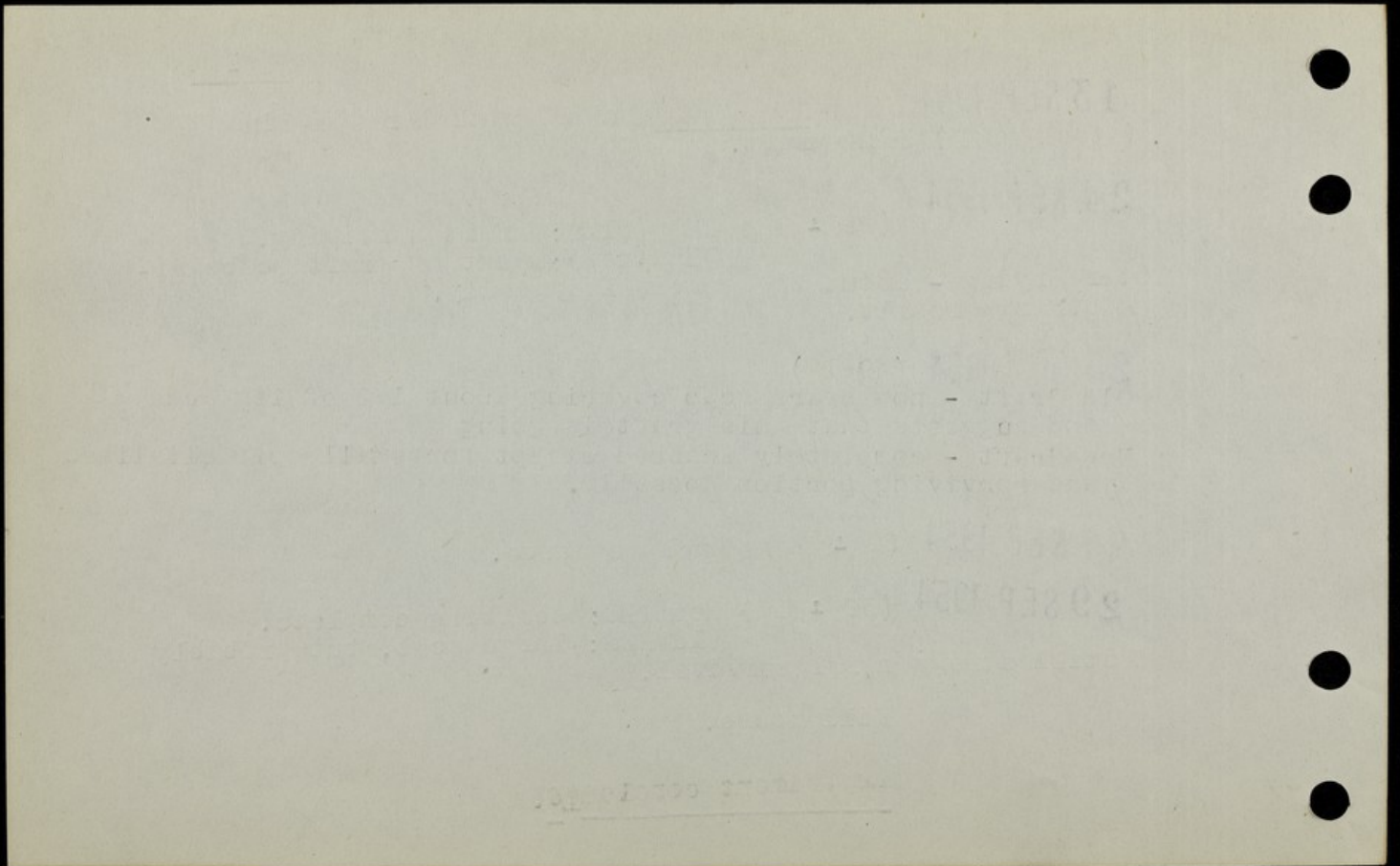
27 SEP 1954 (59 ± 14) Much as above.

29 SEP 1954 (59 ± 16) New LHS: scabbing complete.
Old RHS: large scab, but probably
still some ventral survival.

1.9 OCT 1954 Kill mouse.

Experiment concluded.

scab
O graft
margin



TRANSPLANTATION OF TOLERATED TUMOUR GRAFTS TO
A-LINE & CBA-LINE ADULTS

TUM-16 A,B,C

13 AUG 1954 DONOR MATERIAL: Excise surgically, & cut up for trochar implantation in usual way,

- (A) Tumour (9g) tolerated 73 days by CBA mouse TUM-14.
- (B) Tumour (4g) - 71 - - - - TUM-15.1 R.ear
- (C) Tumour (10g) - 71 - - - - TUM-15.2 No mrk

Note that asepsis was most dubious, and the tumours were enormous & extensively necrotic. Nevertheless fresh-looking material (friable in B,C) was carefully chosen, and it should be O.K. barring local infection.

|| Each mouse dose consisted of 5-6 pieces inserted S/C RHS and usually
|| extending forwards between shoulder blades.

- (A) Transplant Tumour A into 3 CBA 20 females & 3 A 19 females (same age)
- (B) - - - B - - - - - - - - - -
- (C) - - - C - - - - - - - - - -

27 AUG 1954 (14)

- 17
- A. CBA's: 1/3 with faintest trace of nodule
2/3 no trace of tumour
A's: 2/3 with tumour nodule
1/3 with hard pin-point nodule
- B. CBA's: 1/3 with hard small nodule
2/3 no trace of tumour
A's: 3/3 with tumour nodules
- C. CBA's: 1/3 with trace nodule
2/3 no trace of tumour
A's: 3/3 with large nodules

27

13 SEP 1954 (31)

- A.B.C. 8/9 A-line recips.; large tumours on point of ulceration.
1/9 " " : no trace of tumour (A.)
- 9/9 cba recips.: no trace of tumour.

Kill all mice.

Result: No change in specificity of tumour.

EF

18 AUG 1954 Donor material: 6th passage UCZ-1 tumour. Take cortical samples of perfectly clean, healthy, well vascularized tissue; cut up with cataract knives. The tissue was friable and most of it broke up into tiny cell clumps passing easily into the wider-bore Agla needle. Suspend in saline.

Hosts (A) - Experimental. Litter of 7 = 10C3/6.1 - 7. These had not been born at 8 p.m. last night; this morning many had not yet been cleaned up. Note 2 stillbirths. Birth probably at 5-6 a.m. = **5 hrs after delivery**.

Inject **0.02 ml** into each, passing wider bore Agla needle forward between shoulder blades rather yo one side of midline. Inject 0.01 first, then partially withdraw needle & inject the rest. Only the smaller clumps went in.

(B) - Controls. Bilateral subintegumentary injections of 0.02 ml in each of 4 A-line ~~Stock~~ 20 females (still small: wts 17-20 g).

NOTE: owing to syringe failure one of these mice (unmarked) got an effective injection on one side only -- just possibly on neither side.

21 SEP 1954 (34) A. 0/7 tumours

B. (controls) 2/4 tumours (large). Kill.

This result is disturbing.

21 AUG 1954 DONOR MATERIAL. 6th passage UCZ-1 tumour (different from TUM-17); also donor to tumour 7th passage q.v., which will act as a fragment size control. Proceed just as under TUM-17.

(A) Experimental. Litter of 8 = 11C6/2.1-8. Not born 10 a.m., injected 3.30 p.m. (say 3-5 hrs after birth). 0.02 ml each

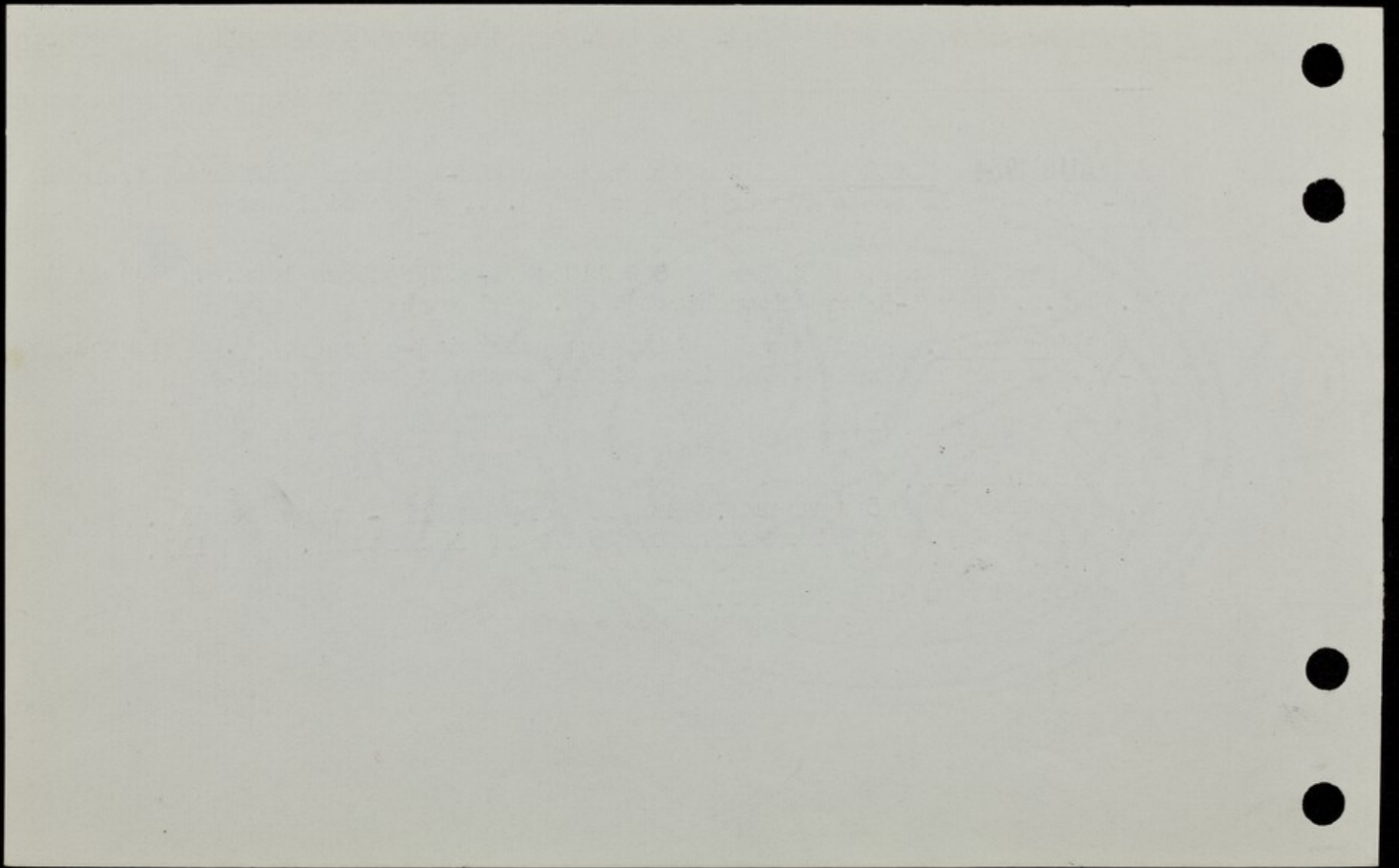
(B) Controls. Unilateral subintegumenatry injections of 0.02 ml each. A-line Stock 20 females. RHS insertions, somewhat posteriorly.

21 SEP 1954 (31)

A. 0/8 tumours.

B. (controls) 4/5 tumours. One is very small - tiny nodule.
All tumours posterior near point of insertion. Kill.

A most disturbing result.



IMPLANTATION OF TUMOUR FRAGMENTS (UCZ-1)
INTO NEWBORN CBA MICE

TUM-19

)))-----

20 OCT 1954 Tumour material: eighth passage tumour -
rather hard and fibrous. Cut up into small fragments.

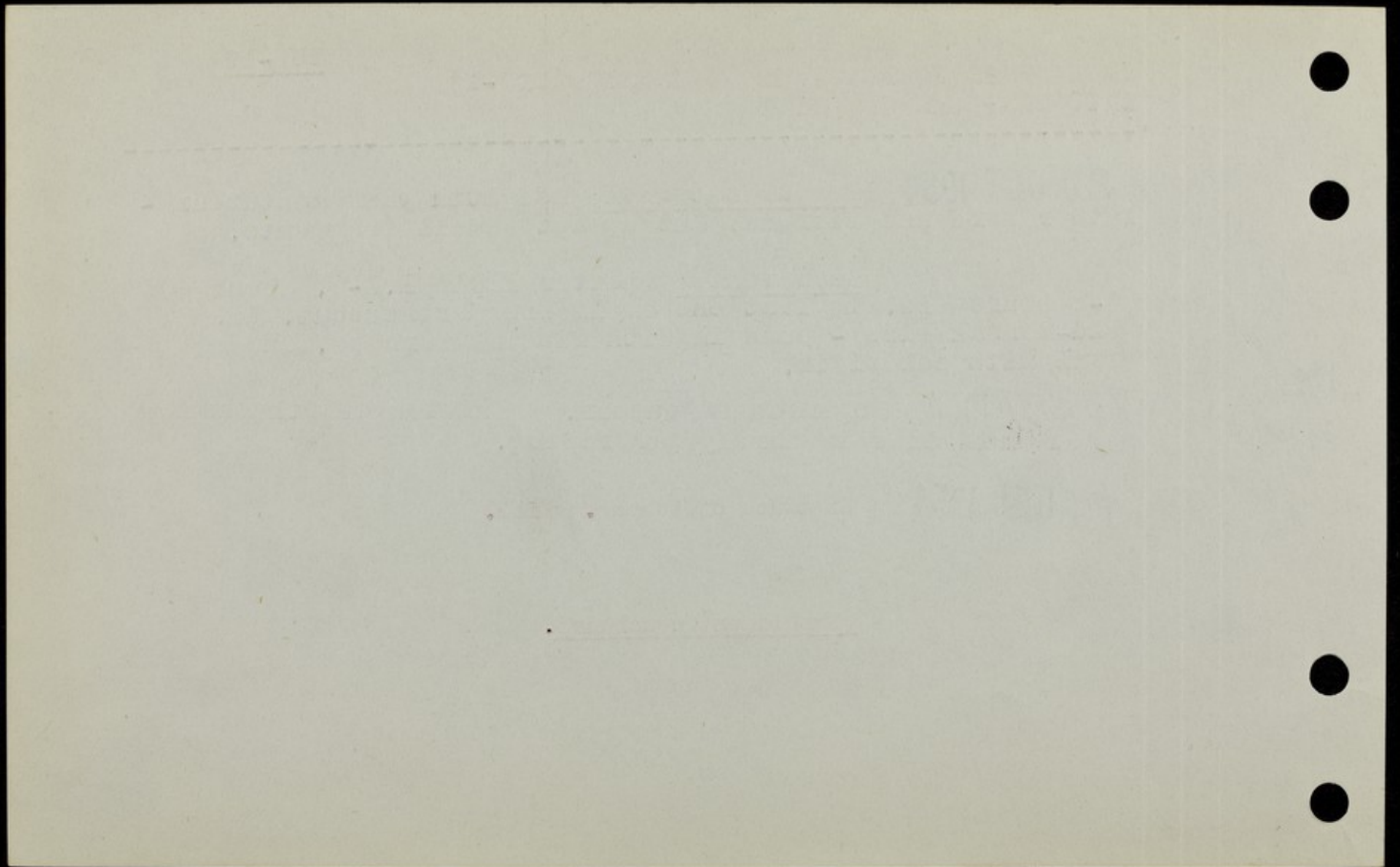
Recipients: litter 11C10/1.1-5, about
4-5 hours old. Implant one small fragment subcut. in
mid-dorsal line - push in with fine MM forceps, and
seal with celloidin.

20 NOV 1954 No trace of tumour. In view of 9th passage
result this is hardly surprising.

15 DEC 1954 No trace of tumour. Kill.

Experiment concluded.

15 DEC 1954



IMPLANTATION OF TUMOUR FRAGMENTS (UC2-1)
INTO NEW-BORN CBA MICE)

TUM-20

20 OCT 1954

Tumour material: as for TUM-19

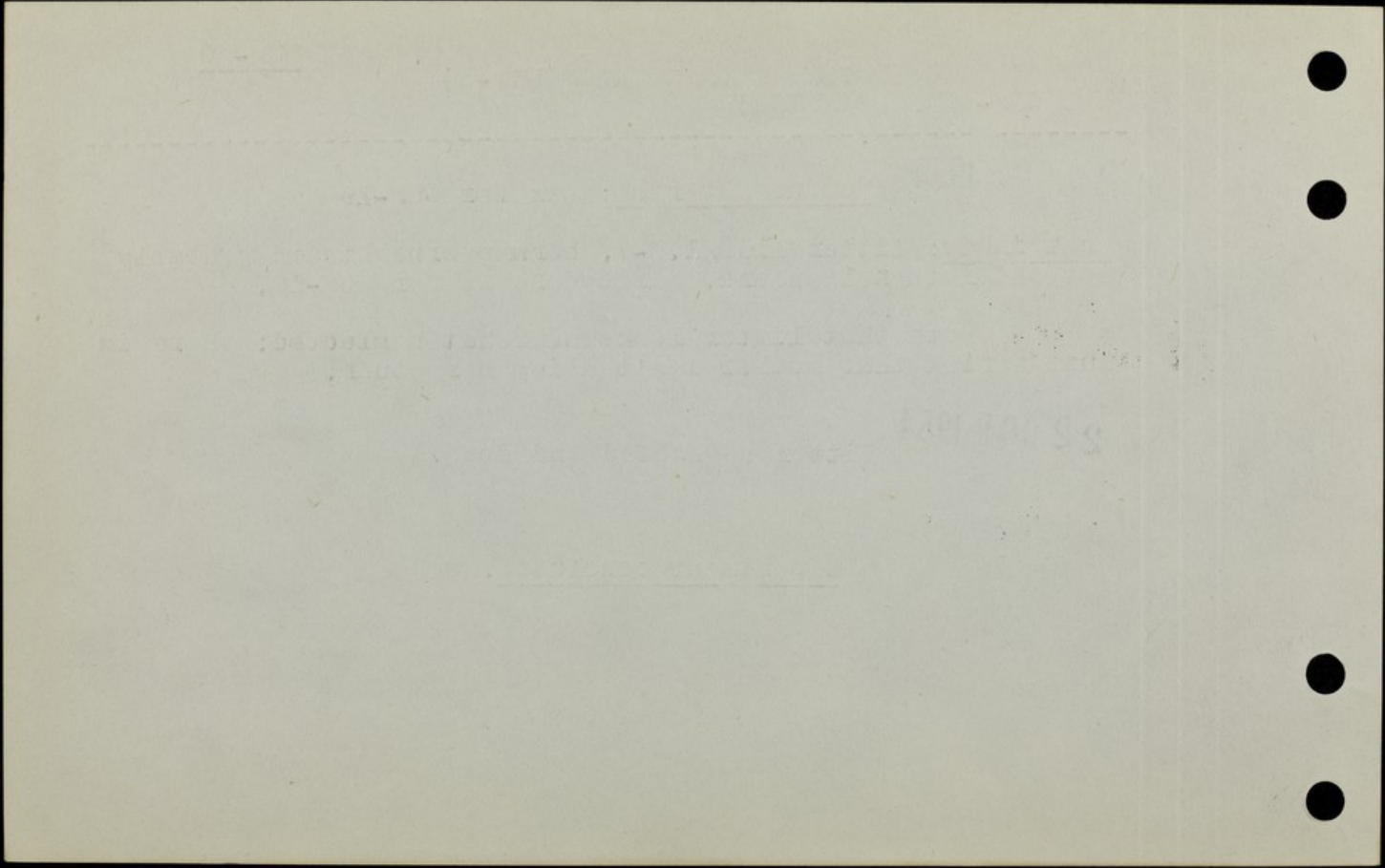
Recipients: litter 11C9/1.1-7, born overnight and probably
not older than 12 hours. Procedure as for TUM-19.

Note that litter looks somewhat neglected; there is
just a risk that mother isn't doing her stuff.

22 OCT 1954

Litter neglected and dead.

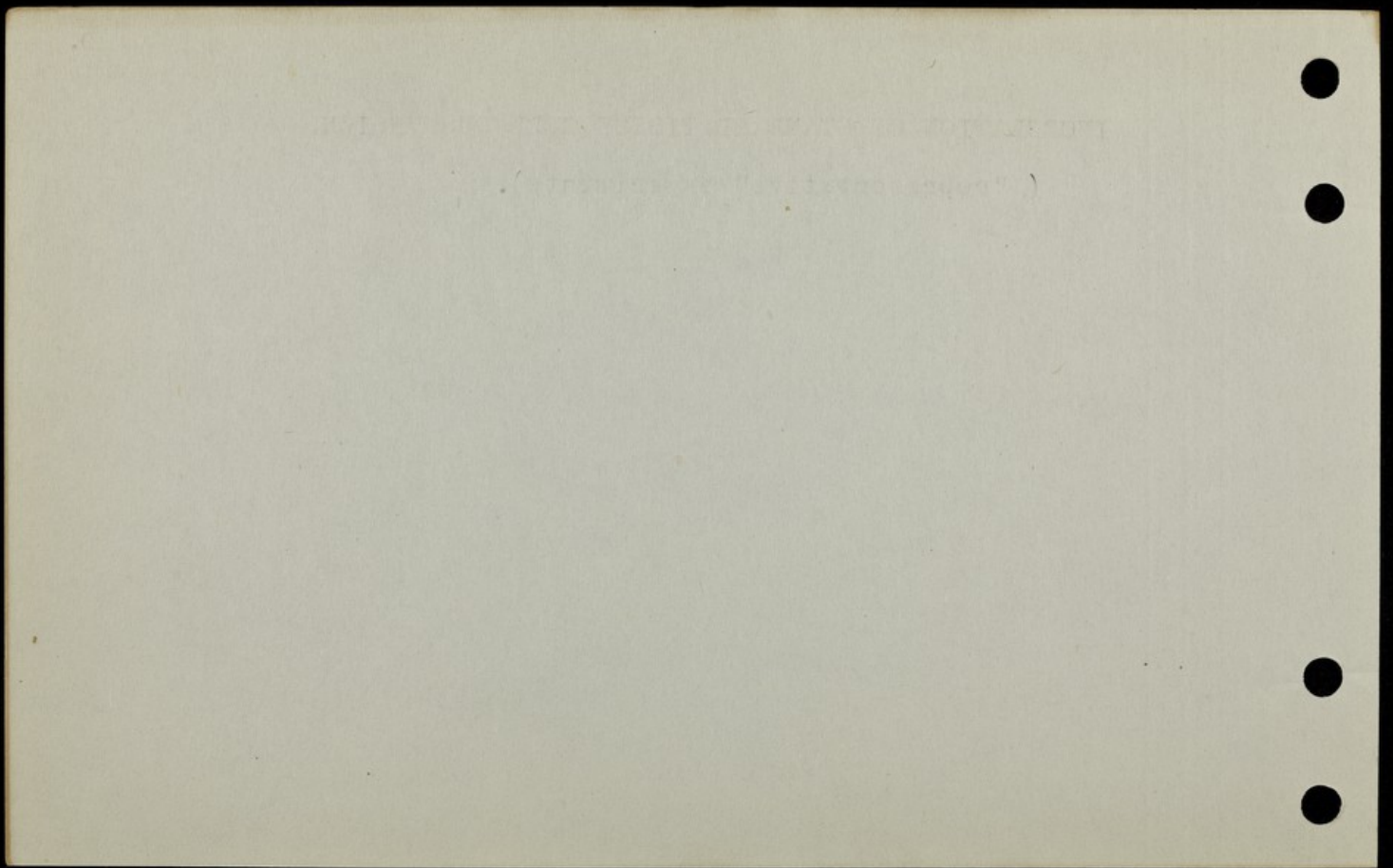
Experiment concluded.



D.

INOCULATION OF STANDARD TISSUE CELL SUSPENSION.

("representative" experiments).



INOCULATION OF FOETAL MICE (CBA to A) WITH
ADULT TISSUE CELLS

EMB-246

14 JUL 1954 Donor tissue: As for EMB-245.

Recipients: Foetuses of 10A1/1 female (ex-cross test).

Inject 7 embryos, 16-17 days old. 0.01 ml.
4 of the injections really satisfactory - rest mediocre.

18 JUL 1954 Litter of 4 born overnight. Age about 16 days
on day of injection. Not unhopeful.

27 AUG 1954 Test-operation.

Donor: Stock 21 cba male. Standard grafts. RHS ops. on
all 4 mice.

6 SEP 1954 (10)

4/4 with 100% survival. Note that mouse with best-
looking graft died under nembutal anaesthesia. Remove
graft as slot: specimen EMB-146.4.

PTO

Number mice as follows:

1. R Graft in good shape
2. L " " "
3. No Graft O.K., rather small
4. - Mouse dead. Graft looked excellent and would almost certainly have been super.

8 SEP 1954 (12) 1. Graft uncontracted and in good shape. Pigmentated.
2. Ditto.
3. Graft going through weak reaction - light criss-
cross scabbing; but as yet high degree of survival, and patchy pig-
mentation.

13 SEP 1954 (17) 1. and 2. Grafts growing good crops of hairs.
Perfect.
3. Graft has suffered some contracture, but seems to
have recovered from reaction and is growing hairs.

18 SEP 1954 (22) 1 and 2. Excellent. Bushy furs.
3. Recovery complete - dense fur on contracted graft.

30 SEP 1954 (34) ditto.

23 OCT 1954 (57) IMPLANTATION OF NORMAL NODE SUSPENSION.

Colour-photograph all 3 animals - with hair-crops and after clipping. (No.3 after clipping only).

Grafts in excellent shape - no blemish of any kind. Nos.1 & 2 large, 3 rather small.

Approx. sizes: 1. Dv: 12 mm. A/P: 10 mm
 2. 11 " 7 "
 3. 6 " 6 "

Remove axillary, inguinal, and 2-3 cervical lymph nodes from 7 mice (11A4/P, 11A2/P, 11A1/P and 11A3/P males, and 3 stock 25 males). Total: 57 nodes.

Nodes generally rather small. Remove fat, and express through fine steel sieve - 3 washings. Make up volume to 3 ml, and inject i/p 1 ml into each mouse. Suspension looked very milky.

Each mouse received the equivalent of 19 nodes.

8 NOV 1954 (57 + 16)

1. Graft completely healthy; 12 x 10 mm; marginal hair-regeneration in progress.
2. A few small superficial scabs have appeared, and rest of epidermis a little scurfy. Hair-regene-

ration in progress; no contracture. Too early to say whether scabs are specific.

3. 2 small marginal scabs; otherwise graft looks healthy enough; quite good hair-growth has taken place. No contracture.

11 NOV 1954 (57 ± 19)

1. No signs of reaction. Hair-growth now in full swing.
2. Not much further hair-growth; multiple small scabs, scuffy surface; 10 mm x 6.5 - slight contracture.
3. Further though slight deterioration - pronounced ventral and anterior scabs, and rest of surface slightly scaly. Close-clip hairs which are fairly long. 6 mm x 5 mm - slight contracture.

19 NOV 1954
(57 ± 27)

1. No sign of reaction. New bushy fur.
2. Graft bald, scabbing, severe contracture. About 1/4 or less of original graft surviving.
3. Breakdown just about complete.
Score: 57 ± 27

23 NOV 1954

- (57 + 31)
1. No sign of reaction.
 2. Breakdown nearly complete - survival slight.
- Colour-photograph 2 and 3.

26 NOV 1954

(57 ± 34).

1. Ditto.

2. Breakdown complete.

Score: 57 ± 34)

4 DEC 1954 (57 + 42) 1. Clip graft area - removal of bushy fur revealed slightly scurfy but otherwise healthy epithelium. One tiny scab. Dimensions: 11.5 mm x 9.0 mm.

6 DEC 1954 (57 + 44) 1. Several scabs have appeared post., ~~dorsal and ventrally~~ dorsal and ventrally. 10.5 mm x 8. Possibly slight contracture??

This might be the beginning of a specific reaction. Watch.

Re-operation of 2 and 3. (second stage grafts).

Donor: 11C3/P. male. Standard celloidin grafts. LHS opns.

14 DEC 1954 (8-day inspection of 2nd stage grafts):

2. Breakdown far advanced; about 25% survival.

3. Breakdown complete.

1. Old graft has scabs dorsally and ventrally, is going bald, and with scurfy cuticle. $10\frac{1}{2}$ x $7\frac{1}{2}$ mm - no significant contracture.

15 DEC 1954 No. 2 has also completed breakdown.

Conclusion: both ns. 2 and 3, having received non-immune node cells, are now immune.

23 DEC 1954 [57 + 67].

246.1 Still in process of the delayed chronic reaction which began 4-6 DEC, i.e. at 57 + 42/4. At present 25% survival approx with wispy hairs still present. FOLLOW.

[As it stands this result need mean nothing; but note that all contemporary supermice of the group EMB-240/5/6 are OK except where grafts have been deliberately knocked out.]

246.2 Destroy
246.3

31 DEC 1954 [57 + 75]

246.1 Further deterioration: graft surface contracted, scaly, eczematous; but still some survival.

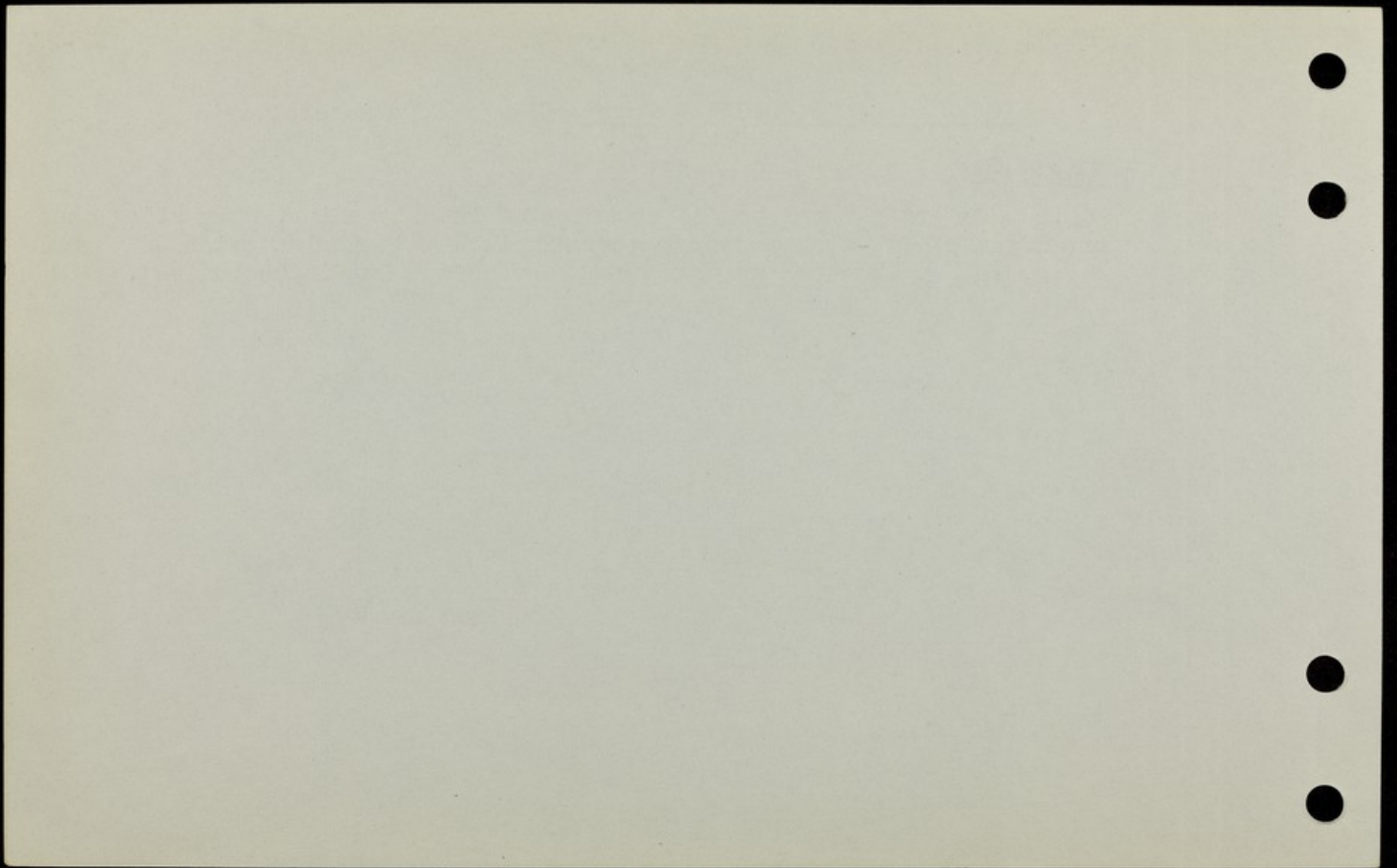
5 JAN 1955 [57 + 80].

246.1 Breakdown is now certainly complete, having begun at 42/4 days. It cannot be held certain that the reaction was due to the implanted nodes, but comparison with contemporary supermice makes it at least possible.

SECOND STAGE OPERATION. Donor: CBA 30 F, quiescent skin.

13 JAN 1955 [57 + 80 + 8 = 145].

246.1 New graft shows **total breakdown of long standing**, suggesting that breakdown of 1st-stage graft was genuinely due to implantation of normal nodes, rather than to 'spontaneous' regression (but inconclusive).



INOCULATION OF FOETAL MICE (CBA to A) WITH ADULT
TISSUE CELLS

EMB-245

14 JUL 1954

Donor: Stock 17 cba male. Prepare usual very dense suspension by chopping method. Liver, testis, kidney and spleen.

Recipient: Accredited A-line inbred.

Inject 8 foetuses, about 16 days old. 0.01 ml. Injections very satisfactory - vis. good. 1 injection probably into head region.

18 JUL 1954

Litter born overnight. 4 youngsters. Age at injection 16 days. Hopeful - see notes.

27 AUG 1954

Test-operation.

Donor: Stock 21 cba male. Standard grafts. RHS opns. on all 4 mice.

30 AUG 1954

Test-operation.

Donor: Stock 21 cba male. Standard grafts. RHS opns. on the 3 survivors.

9 SEP 1954 (10) All three grafts show 100% survival. Yield 3/3.

- (14) 1. (R ear mauled) Uncontracted, still a little delicate, dorsal hair-growth.
2. (L) Very slight marginal scabs, graft rather swollen but also intensely active. Hair-growth over large part.
3. (no) Very light pigmentation; faintly contracted; graft rather small from start. Incipient hair-growth.

18 SEP 1954 (19) All 3 grafts are doing very well and growing crops of hair - perhaps a little patchily.

30 SEP 1954 (31) All 3 grafts in fine form; good hair-crops.

27 NOV 1954 (90) Ditto.

4 DEC 1954 (97) All 3 grafts have good hair-crops. Clip and inspect.

1. 10 mm x 9. Graft perfect except for two tiny fresh scbs posteriorly.
2. 11 mm x 5. Graft perfect, but intensely active.
3. 8.5 mm x 8. Graft perfect. A few white hairs present.

6 1954

1954 MAP 412

6 DEC 1954⁽⁹⁹⁾ ORTHOTOPIC INOCULATION OF NORMAL NODE CELLS.

[A] From each of two A-25 FF take 4 axillary, 2 inguinal & 2 cervical nodes: total 16, and nodes of good size. Squeeze through sieve into the minimal quantity of normal citrate saline and take into $\frac{1}{4}$ ml tuberculin syringe through very finest needle. Total vol 0.15 ml, and perhaps containing the cellular matter of 8 nodes (allowing 50% loss). The suspension, though exceedingly dense, was perfectly stable.

[B] Expose the (draining) RHS axillary nodes of EMB-245.1 by the usual incision; mobilize just sufficiently to get a good presentation without cutting any important vessel or pulling up from bed. Inoculate each with normal node suspension through several puncture insertions. The exact volumes that entered are uncertain, but the superficial axillary (= brachial node) blew up prodigiously; the deep axillary was also inflated, though not nearly to the same extent, and there was considerable leakage. Some only of the leaked matter remained in situ, and will probably simply disappear. Nevertheless, this was a promising experiment and shows the feasibility of the operation. In future, cut down fluid still more.

[EMB-245.1. R ear (nibbled), EO head. Graft still 10 x 9, and except for the two small blemishes noted at 97 is still perfect.]

--See also 240.4 for notes on AU immunization exp.

11 DEC 1954 [99 + 5] So far from deteriorating, the tiny scabs on the graft have healed right over, and much of the graft is now active, with 2-3mm new hair growth.

13 DEC 1954 (99 + 7) 1. (Intra-node transfer): graft intensely active; dense hair-crop - clip. One or two very minor scabs and slight swelling. 8.25 x 9.5 mm - slight contracture.

2. Graft perfect except for tiny healed scab posteriorly.

Good hair-crop. Clip.

3. (SERUM): 'immune' serum injections began 5 days ago. Graft perfect - not the slightest sign of a reaction.

21 DEC 1954 [99 + 15]. 245.1 (normal nodes). There has been further hair growth since 99 + 7, but graft is no longer active. Clip hairs. Graft shows no definite pathological change, though there is a suggestion of hardening round periphery.

23 DEC 1954 [99 + 17 = 116].

245.1 [normal nodes] [EO, R ear]. Normal graft: no deterioration, so that appearance at 99 + 15 cannot be taken seriously.

245.2 (L, no colour); Still tiny dorsal scab but fair graft.

245.3 (O, gentian) [SERUM]. Further hair growth: perfect graft.

26 DEC 1954

245.1 [99 + 20]. Normal nodes. Surface has minute almost epithelial scabs, but graft is soft and hair pelt is normal. No significant deterioration.

31 DEC 1954 [124].

- 245.1 [99 + 25] Normal nodes. Definitely no further deterioration. Graft looks reasonably good.
245.2 Graft is active: tiny marginal scabs.
245.3 (Serum) Graft O.K.

4 JAN 1955 [128].

- 245.1 [99 + 29]. Normal nodes. Graft still looks reasonable good; is uncontracted: in quiescent state.

11 JAN 1955 [135].

- 245.1 [99 + 36]. Normal nodes. Graft is now slightly contracted & very scaly. Hair growth has not progressed. ?Reaction.

17 JAN 1955 [141].

- 245.1 [99 + 42] (Normal nodes, orthotopic) Severe dorsal & ventral scabbing, but central narrow strip of surviving epithelium.
245.2 (normal). Contracture & some scabs, but a few hairs & high survival
245.3 (serum). Breakdown was complete about 4 days ago, viz: at 137 days.

22 JAN 1955 [146].

- 245.1 [99 + 47]. Hard to interpret. Graft now looks like a straight

resurfaced collagen pad. PBM thinks breakdown was complete well before 99 + 42, notwithstanding note. But there are hints of pigmented hair follicles: if these come to live, it can only be assumed that breakdown fell just short of completion. If not, breakdown can probably be taken at about 99 + 39.

29 JAN 1955 [153].

245.1 [99 + 54]. No trace of pigmentation; graft area looks like old scar.

10 FEB 1955 2. (164) Graft now reduced to a small remnant bearing a few hairs.

20 FEB 1955 2. (174) Breakdown complete. Score approx. 174 days.

EB 1955

1955

EB 1955

1955

11 JUL 1954 DON OR:- CBA stock 20 (young) male. Spleen, 2 testes, $1\frac{1}{2}$ kidneys, piece of liver chopped almost to completion with scissors under normal saline; concentrated. Particles of size to pass wider bore needle were used. All fetuses received many large organized tissue lumps as well as finer matter.

NOTE:- 'A-line inbred female' means an unmarked female belonging to the special stock-pots set up for EMB operations. All these mice are from accredited line litters.

- 237. 8A3/3 or 10A3/1. 6 x 17-day. More present. Injections good.
- 238. A-line inbred. 7 x 16-days. More present. Injections moderate; 1-2 very uncertain.
- 239. 8A3/5. 8 x 16-days. Probably 1 more present. Vis. only moderate: some injections poor.
- 240. 10A6/2. 8 x 18-days. Injections very good.
- 241. A-line inbred. 9 x 16-day. Vis excellent, good injections.
- 242. A-line inbred. Too young: discard.
- 243. 8B5/1. 4 x term. 2 x 0.01 ml each. Body wall cut: 3 stitches. injections certain. 5-6 present. Prognosis hopeless (see below).

244. 9B2/4. 7 x 15-day (more present). Expose through deliberate body-wall incision (2 cm long: close 3 stitches). At least 4 injections **excellent** for this age.

NOTES: All fetuses received 0.01 ml exc. 243 & 244 q.v.
Wide bore needle throughout.

FOLLOW UPS

- † 237 14/15 3 + (3 dead) 16th: All dead.
- † 238 14th: p.m. 2 born. 17th All dead.
- † 239 13/14 2 + (1 dead) 18th: All dead.
- 240 12/13 - 8 born: 2 have hemorrhagic marks. 22nd: 7 ✓
- † 241 16th: Animal distressed, abnormal posturing, had to be killed.
- ~~242~~
- 243 Still birth amongst 11/12 July - born a few hrs after operation.
- 244 15/16th: Litter of 5. 22nd: 4 mice (incl. 1 runt)

30 AUG 1954 Test-operation.

EMB-240

Donor: Stock 21 cba male. Standard grafts. Operate all 7 mice.

This should be quite a hopeful expmt, though a little late.

9 SEP 1954 (10) INSPECTION. 1/7 = 0, typical total breakdown. 6/7 = 100% survival, two however showing some reddish patches. Note that these are very large grafts. No dressings.

13 SEP 1954 (14)

1. (R) Graft ~~is~~ a little swollen but intensely active - dense hair-growth.
2. (L) Tiny (non-specific) scabs healing; no contracture; hair-growth in progress.
3. (no) As for 2.
4. (both) As for 2.
5. (2R) Very slight contracture and scabbing; ep. lacks matness; patches of faint pigmentation; no hairs.
6. (2L) As for 5, but more extensive scabbing.

18 SEP 1954 (19)

1. Graft intensely active; dense fur (a few white hairs in centre). Slightly palpable - may be due to activity.
2. Graft fine with cba hairs - a few white hairs in centre.
3. Graft fine. Small patch of white hairs in centre.
4. Graft fine - dense fur. Small (non-specific?) scab dorsally.
5. About 75% survival. A few hairs. Small scabs.
6. About 50% survival. Slight hair-growth.

30 SEP 1954 (31)

1. 2, 3, and 4 are in fine form.
5. Almost wholly surviving; little contracture; a few minor and superficial scabs; graft pretty bald, but has patches of wispy and sparse hairs.
6. Only small remnant surviving; scabs; contracture. A few hairs betray survival. Graft has steadily and slowly deteriorated.

9 OCT 1954(40)

- 1-4 are doing well; but note high proportion of unpigmented hairs in the grafts.
5. Wispy hairs still present, but superficial scabbing continues & there has been considerable contracture.
6. Breakdown can be taken as complete some time ago: say at >30 <35 days.

27 NOV 1954 (89)

- Nos. 1, 2, 3, and 4: excellent form.
5. Further contracture; graft remnant now very small, but still a few cba hairs..

4 DEC 1954 (96)

1. 12 mm x 6. Graft intensely active and perfect except tiny scab ventrally. A few white hairs.
2. 11 mm x 9. Quite a number of white hairs. Perfect except for tiny scab in centre.
3. 12 mm x 9. Tiny scab on dorsal margin, but likely to be nonespecific since host skin has similar defects. Some white hairs.
4. 11.5 mm x 7.5. Perfect. A few white hairs.
5. Tiny graft remnant; but it is still pigmented and bears a few cba hairs.
6. Nothing. See above.

6 DEC 1954 (98) Reoperation of No.6 (second stage graft).

Donor: 11C3/P male. Standard celloidin grafts. LHS opn.

[Graft 8 AU males, ex stocks 6,7 with standard RHS CBA grafts from St 26 FF, in preparation for 'AU NODE EXP' on 240.4 & 245.2]

8 DEC 1954

~~8~~ 14 DEC 1954 (100) Use no.1 for serum experiment: injections of immune serum begin to-day. Graft perfect.

13 DEC 1954 (105) 1. (SERUM) Perfect.
2. Now non-active. O.K. except for shiny healed scab in centre.
3. Most of graft O.K., but prominent central ulcer.
4. Graft perfect except for tiny scab ventrally.

16 DEC 1954 (108) 1. (SERUM) Perfect
6. (2nd stage graft - 10 days): 100% survival. Graft in pretty good form.

16 DEC 1954 IMMUNIZATION OF A-SUPERMOUSE ANTI-CBA WITH AU NODES [EMB.240.4].

(A) Take 5 x 2 regional axillary nodes from 5 AU male mice grafted 10 days ago with CBA skin graft. Nodes of middling size only. Press out node contents by sieve method, and take up in total (3 washings) of 1.0 ml normal saline.

(B) Inoculate 240.4 I/P with the AU (anti-CBA) immune node cell suspension.

[Object of exp is to break down the CBA-on-A tolerated graft with a node inoculum which will itself break down: and then to do a second-stage CBA graft. If the specificity is absolute, the 2nd-stage graft should take. Present graft is OK except for persistent tiny epithelial blemishes which may not be specific.]

21 DEC 1954 240.4 [108 + 5]. (Nodes from AU mouse.) This graft is pretty generally covered by tiny puckering scabs, and may also be a little inflamed. This represents a marked deterioration since the day of implantation, and definitely represents a graft on the way out. Whether deterioration will go to completion remains to be seen.

23 DEC 1954 [115].

- 240.1 (R; blue). Perfect graft [serum].
- 2 (L) No treatment. OK apart from greying and tiny central defect. Active.
 - 3 (O) No treatment. Central defect healing. Good graft.
 - 4 [108 + 7] (Both ears; PIC). Graft no worse than at 5 days; tiny puckered scabs still there, and definite contracture. [AU NODE EXP].
 - 5 (2R) No graft remains. Take breakdown time as 110 days. REGRAFT.
 - 6 (2L) **SECOND STAGE GRAFT**. Breakdown was complete at 98 + 13/14 days.

26 DEC 1954 [118].

240.4 [108 + 10]. No further deterioration.

29 DEC 1954 [121].

240.4 [108 + 13]. Slight deterioration since +10, in form of contracture and smooth rather shiny surface. A growth cycle may be incipient.

31 DEC 1954 [123].

240.1 (serum). Perfect

240.2 & 3 (normals). Good grafts with renewed hair growth.

240.4 (AU NODES) (108 + 15). Although definitely reduced in size the graft is now **active** and deeply pigmented; new hairs piercing from dorsal half.

THUS THE AU IMMUNE NODES HAVE NOT BROUGHT ABOUT BREAKDOWN:
ONLY A TEMPORARY SETBACK.

4 JAN 1955 [127].

240.4 (AU NODES) (108 + 19). Graft is now densely furred: only evidence of its temporary setback is the fact that it has contracted slightly.

5 JAN 1955 [128]. SECOND STAGE OPERATIONS on 240.4 [108 + 20] and on 240.5 ('spontaneous' breakdown after long drawn-out reaction, timed at 110 days). Donor: CBA St 30 F, quiescent skin.

13 JAN 1955 [128 + 8].

240.4 (AU nodes). Old (RHS) graft OK. New (LHS) graft 100% survival.

240.5 (Straight 2nd-stage graft only). Trace survival: score 8 days.

15 JAN 1955 [128 + 10].

240.4 (AU nodes) New LHS graft has deteriorated rapidly over past few days: now ~~25%~~ survival. Old RHS graft though haired has in fact contracted & scabbed, and shows about same degree of survival.

240.2 (Normal) Graft active: two small scabs. A little suspicious.

15 JAN 1955 [138] REPEAT OF AU NODE EXPERIMENT ON 240.1 & 240.3.

Take 32 axillary nodes from 8 normal AU mice bilaterally grafted 10 days ago with standard CBA skin grafts. Prepare 2ml node suspension in Ringer by usual sieve-and-pestle method. Nodes were not large. Inject each mouse with 1.0 ml intraperitoneally:-

240.1 R ear. Blue [ex-Serum exp., v.s.]. Graft perfect & active. 13mm DV X 6mm AP.

240.3 O ear. Eosin. Graft in fairly good shape: small antero-dorsal scabs. Non-active. 10mm DV x 5½mm AP.

17 JAN 1955 [140]

240.2 (1 ear: Normal). Scabs have healed; graft active.

240.4 [128 + 12: AU NODES] RHS: two small scabs; graft bald exc. at extreme anterior end, but what remains is firmly epithelialized.

LHS (new graft): very severe contracture, but graft remnant appears to be pulling round.

22 JAN 1955 [145].

240.1 [138 + 7][AU nodes]. R, blue. This graft quite perfect.

240.3 [ditto] O, Eo. Graft shows a just perceptible redness & scaliness: most doubtful if this amounts to a definite reaction, as graft was not perfect to begin with. Watch.

240.4 [128 + 17] [First AU node exp: regraft LHS] RHS (old) graft has scar-like appearance, but with suggestion of new pigmented follicles. LHS now really does look as if it had gone, being a small dried scab.

29 JAN 1955 [152].

240.1 [AU NODES: 138 + 14]. R ear, blue. Perfect graft.

240.3 [AU NODES: 138 + 14]. O ear, eosin. Slight contracture & graft a bit scruffy, but essentially normal; some very slight setback could be attributed to AU immune nodes if recovery proves to be complete.

240.4 [AU NODES: 128 + 24]. Both, Pic. LHS (new) graft a total loss. RHS graft shows dim signs of a recovery of pigmentation; but this was visible 7 days ago, & if there were anything in it, new hairs would be expected by now.

10 FEB 1955 (164)

1. Graft perfect. (AU nodes at -26)
2. Very scruffy, but partly haired. (normal)
3. Perfect (AU nodes at -26)
4. RHS graft remnant bearing a few cba hairs. LHS went long ago (see above)
(AU nodes -36)

4 MAR 1955 (186)

1. Graft perfect.
2. Graft remnant with a few cba hairs.
3. Smallish graft with fair hair crop.
4. Breakdown complete. Score approx. 175 days.

TRANSPLANTATION OF TOLERATED CBA GRAFTS TO NORMAL ANIMAL OF HOST STRAIN.

- A. Donor: 240.1. Remove graft as pinch, scrape off panniculus and divide into 2 equal parts. Grafts smallish.
Recips. 2 stock 31 A-line females. RHS.
Replace the removed tolerated graft with a fresh cba graft - donor 11C9/P female. Recip. is pregnant, making bandaging rather tricky.
- B. Donor: 240.3. Remove and trim as above, but transplant as a whole. Note that graft is smallish and dermis appears to be badly fibrosed.
Recips. 1 stock 31 A-line female. (Eosin on plaster).
Replace the removed tolerated graft with fresh graft as above.

14 MAR 1955 (186 + 10)

Grafts on normal hosts: 100, 90, 90%

- 240.1. Graft has healed well, but posterior gape has caused mild infection. Graft unaffected. Perfect.
3. Healing good. Graft in good shape.

15 MAR 1955 (186 + 11)

Grafts on normal hosts: 50, 50, 10%

240.1. Graft fine.

3. Not completely happy; faint marginal scabs, and just perceptibly delicate.

MAR 1955
16 MAR 1955 (186 + 12)

Grafts on normal hosts: 25, 0, 0.

17 MAR 1955

Grafts on normal hosts: 10, 0, 0. Kill. SCORES: 12, 12, 14

240.1. Graft in fine form; pigmented, incipient hair-growth.

240.3. Slight marginal scabs appear to be healing; graft stabilising?

21 MAR 1955 (186 +17) SPECIFICITY TEST.

1. Graft in good shape; but note tiny ventral marginal scab. Hair-growth vigorous.
3. Graft in moderate condition, somewhat contracted and still with a faintly scabby margin. Impression is that tolerance is not quite complete.

Transplant to each mouse (LHS) an AU graft from stock 10 donor.
Protect the established graft with tulle.

29 MAR 1955 SPECIFICITY TEST. (203 + 8)

1. AU graft: b.d. complete and of some standing. Probably immune - due to prior injection of AU nodes.
CBA graft: densely furred - clip. Graft intensely active and appears to be in fine shape.
Complete specificity.
3. AU graft: b.d. has begun, but still high degree of survival. Consistent with MST - 1.
CBA graft: well haired and appears to be O.K.

30 MAR 1955 (204 + 9)

1. CBA graft: in good shape.
2. AU graft: b.d. complete
CBA graft: perfectly O.K.

Complete specificity.

2 APR 1955 (204 + 9 + 3)

1. Graft O.K., with good hair crop. Tiny post. scab - probably non-specific.
3. Graft O.K.

11 MAY 1955 (204 + 9 + 42)

1. Graft in good shape - thick pelt of hairs.
3. Graft with crop of hairs; small scab.

COMPLETE SPECIFICITY CONFIRMED.

- 1 JUL 1955 (305) 1. Graft perfect
3. Tiny graft remnant with a few hairs.
- 5 JUL 1955 (309) 3. Re-operate on LHS. Note that the old RHS graft, now reduced to the smallest of remnants with a few cba hairs, was a little contracted but with good hair-crop on the 18th April
- 15 JUL 1955 (309 + 10) 3. New LHS graft in fine shape.
- 16 JUL 1955 (309 + 11) 3. Breakdown of new graft well advanced.
(309 + 12) 3. B.d. complete. Score: 309 + 12
1. Graft perfect.
- 12 SEP 1955 ((382) 1. Beautiful RHS graft, but mouse with LHS mammary carcinoma.
Excise a 3.49 g tumour from L axillar region. This was done by careful dissection, and the 4 cm incision was closed by running suture reinforced by collodion. The tumour was not adherent to the body wall, but may have broken into the skin. The axillary nodes were intimately associated with the tumour and had to be removed; possibly some tumour remnants remain here, too close to the subclavian vessels for radical extirpation.
- 22 SEP 1955 (392) Graft perfect. Mouse in very good condition; longitudinal scab over suture line is about to come away. No palpable tumour.
- 29 SEP 1955 (399) Ditto. Scab still attached. No tumour in evidence.

27 AUG 1954

Test-operation

EMB-244

Donor: Stock 21 cba male. Standard grafts. RHS opns. on the 4 survivors.

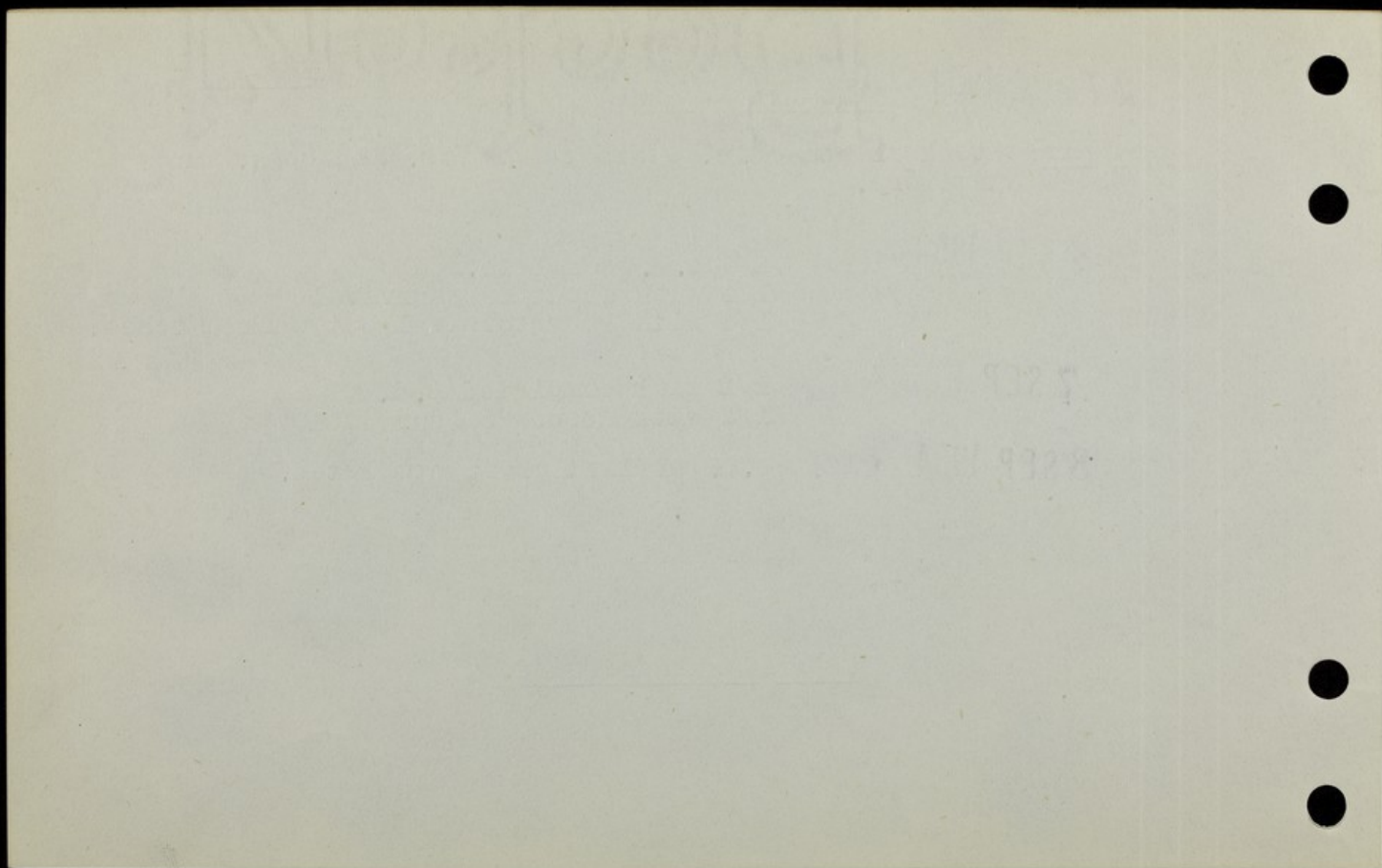
- 6 SEP 1954 (10) 2/4 b.d. complete.
1/4 with about 25% survival
1/4 with 100% survival but weak patches.
- 7 SEP 1954 (11) 1/2 with completed b.d.
1/2 with about 25% survival
- 8 SEP 1954 (12) B.d. of last graft completed.

Results not significant.

Experiment concluded.

78

49



8 JUL 1954 DONOR:- As EMB-234

RECIP:- Foetuses of 8B5/1, possibly o-parous, 33 g.

7 x 17 day old embryos. 0.01 ml. Visulaisation excellent,
and injections very reliable.

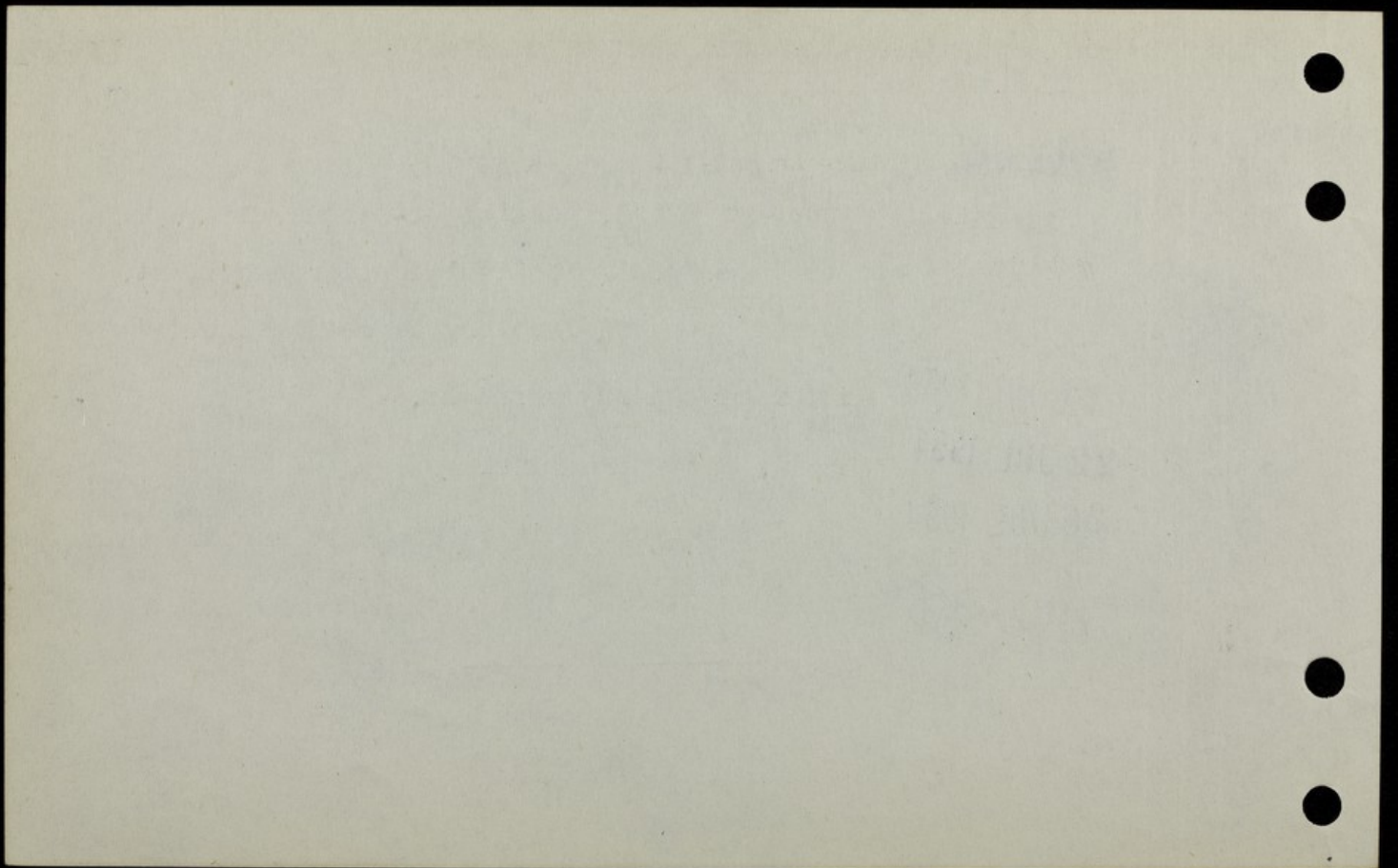
Very hopeful provided recovery is reasonable.

11 JUL 1954 Litter of 4 born overnight.

22 JUL 1954 4 alive, but 2 are runtish.

26 JUL 1954 Only 1 survivor. This is so badly runtish that
it was killed.

Experiment concluded.



8 JUL 1954 DONOR MATERIAL: Exactly as EMB-234.

RECIP:- Foetuses of 9C5/P, 5-parous, 40 g.

5 x 14-15 day old embryos. Too young for reliable injection. Injections variable, always blind, sometimes double. 0.01 ml. One or two received 0.02 ml. Prognosis very poor.

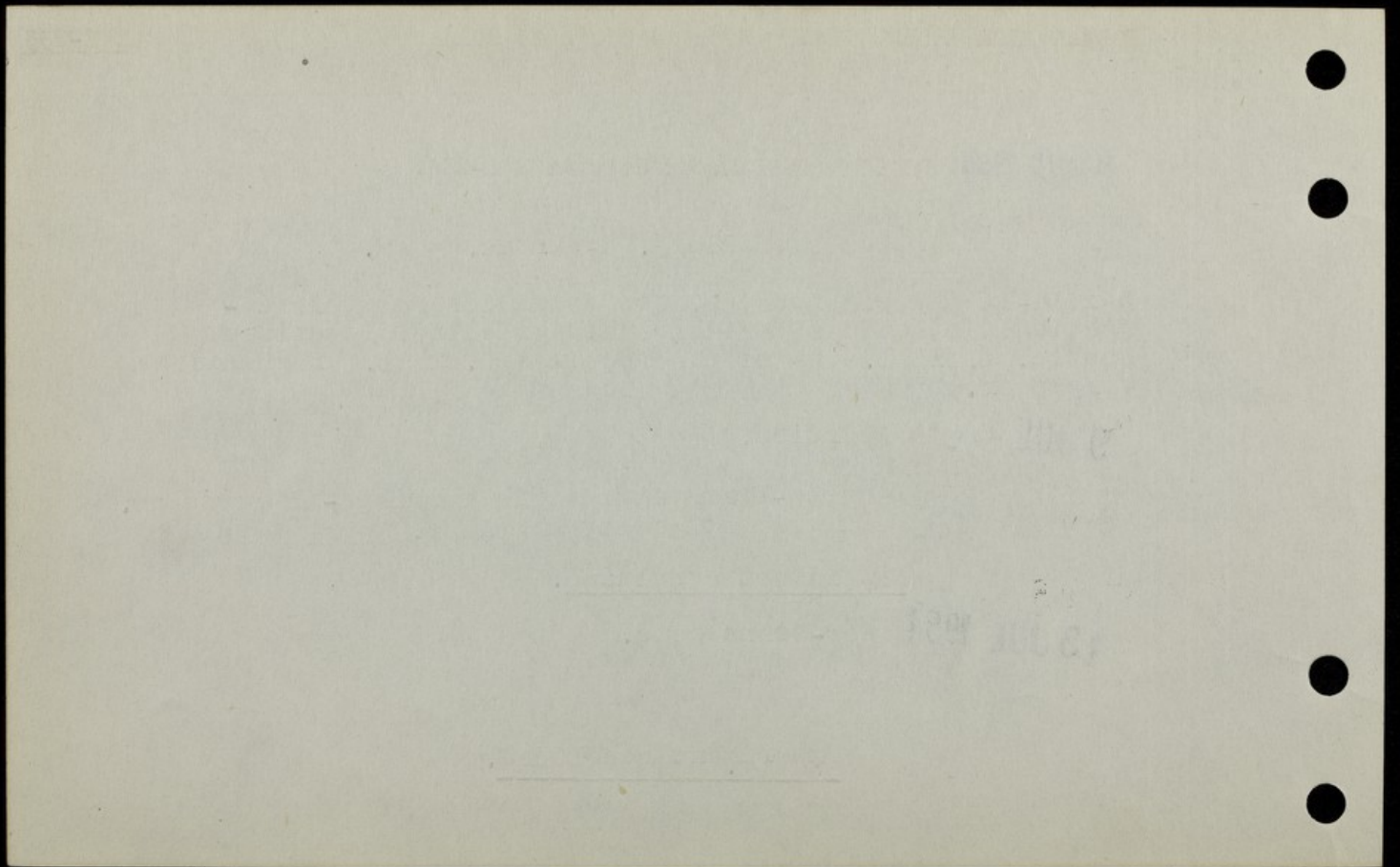
9 JUL 1954 Abortion.

12 JUL 1954 2 new-borns found this morning - 1 is dying.
Experiment pretty hopeless.

Experiment concluded.

18 JUL 1954 New-borns dead.

Experiment concluded.



8 JUL 1954 DONOR:- Stock XIV A-line male. Take $\frac{1}{2}$ kidney, whole spleen, 1 testis, and small piece of liver. Chop up with scissors under normal saline (prolonged chopping necessary). Take up through fine bore AGLA needle, spin down to concentrate, and take up again into needle.

RECIP:- Foetuses of 11C3/P, 3-parous, 39 g.

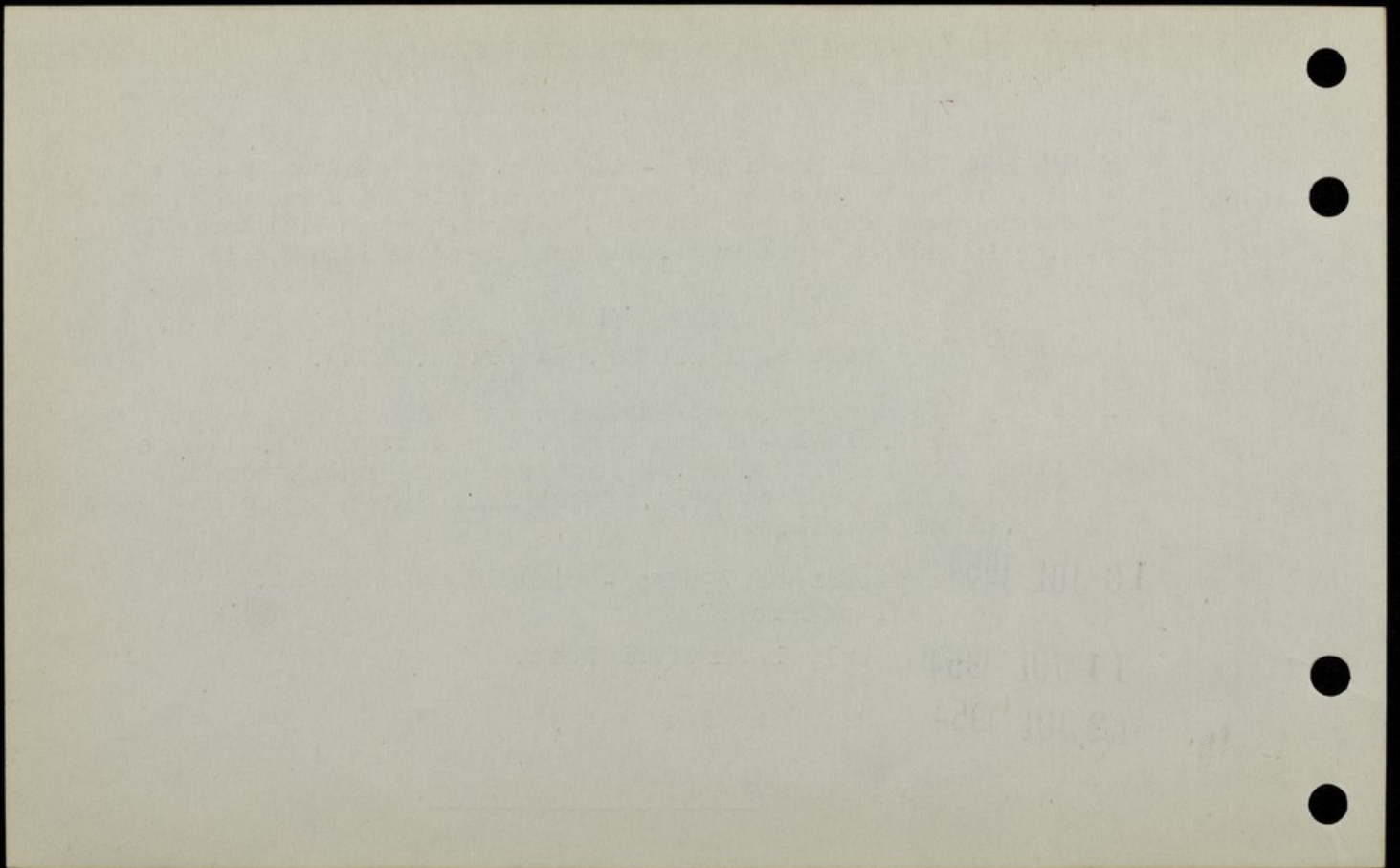
8 x 16 day old embryos, but almost certainly more present. Inject 4 with 0.01 ml - these were done pretty well. 4 more injections rather blind, but 3 of these were re-injected and probably more effectively. Those inoculated twice received 0.01 ml in all.

10 JUL 1954 Litter of 7 born - probably overnight.
Premature???

11 JUL 1954 Only 1 survivor left.

12 JUL 1954 Last survivor dead.

Experiment concluded.



INOCULATION OF FOETAL MICE (A to A) WITH EMB-213
ADULT CELLS.

)))-----

14 MAY 1954

Donor: Exactly as for EMB-210.

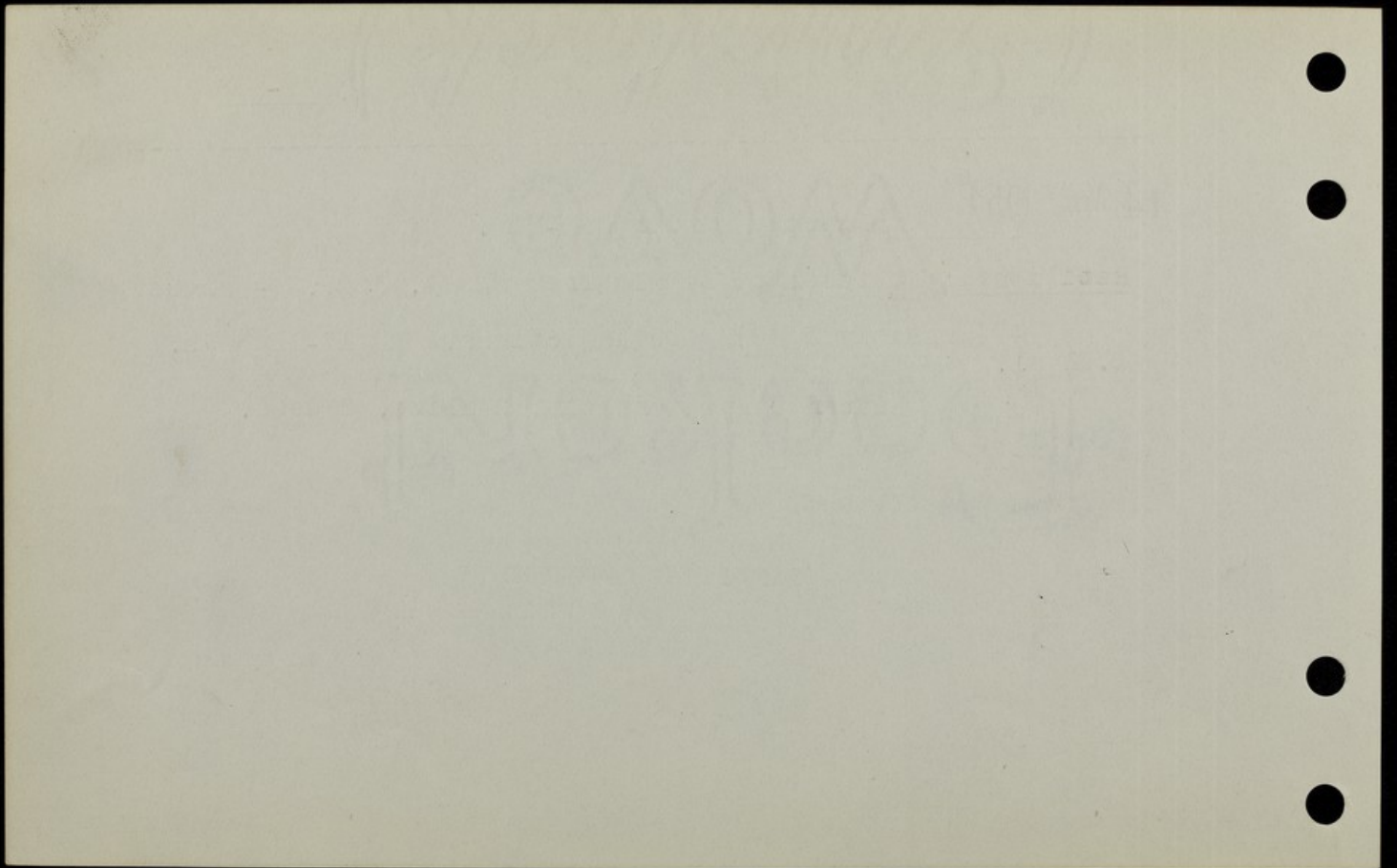
Recipients: Foetuses of female from 8A5/4, 32g., o-parous.

4 (possibly 5 ??) good injections. Age 17-18 days.
0.01 ml.

15 MAY 1954 Litter born overnight. 2 alive, 2 dead.

17 MAY 1954 All dead

EXPERIMENT CONCLUDED



INOCULATION OF FOETAL MICE WITH ADULT CELLS
(CBA to A)

EMB-212

14 MAY 1954 Donor: Exactly as for 210.

Recipients: Foetuses of female from 8A5/4, 35g., 1-parous,
Pieric.

7 very near term. Good injections. 0.01 ml.

15 MAY 1954 Litter born overnight. 8 new-borns.

17 MAY 1954 Six remain

30 MAY 1954 Six remain

16 JUN 1954 4 remain - 2 died 7/8 June. 4

13 JUL 1954 Test-operation.

Donor: Stock 19 cba male. Standard grafts and opns.

23 JUL 1954 (10) 1. (L) Healing sound. 100% survival, but small haemorrhagic and probably weak patches. Rebandage.
2. (R) Healing sound. 100% survival; deep-seated pigmentation over whole surface. Prognosis good. Super? Rebandage.
3. (no) Healing sound. 100% survival, but not quite as promising as 2. Rebandage.
4. (both) Healing sound. 100% survival; anterior margin a little inflamed - probably non-specific. Very open fit. Comparable to 3. Rebandage.

26 JUL 1954 (13) 1. Total b.d. Kill.
2. Graft in excellent condition: heavy pigmentation and hairs are already penetrating. Super.
3. Breakdown in progress; some epithelium. Keep.
4. Graft in very good condition and is lightly pigmented. SUPER.

27 JUL 1954 (14) 2. Hair-growth in full swing.
3. B.D. very nearly complete. Kill. Score: 14 days
4. No change.

28 JUL 1954 (15) 2. Graft in fine form - vigorous hair-growth.
4. Largish marginal scab; rest of graft is lightly pigmented and looks healthy.

31 JUL 1954 (18) 2. Graft in terrific form - for this age hair crop is densest ever seen in this lab.

4. Central scab noted previously spread right across centre of graft, but graft has not deteriorated appreciably in last few days. **On the contrary, the healthy-looking surrounding epithelium is now growing a modest hair-crop.** Rather more than $\frac{1}{2}$ original graft surviving.

11 AUG 1954 (29)

R = 2. Graft perfect exc. for tiny superficial scab DP corner.

Both = 4. Graft just palpable. Very bushy hair crop, but uneven over surface (central scar area, tho contracted, still distinguishable).

16 AUG 1954 (34)

R = 2 (M) Graft perfect

B = 4 (F) Graft perfect. In advanced pregnancy, clearly by 2(M), since 20 days since isolation from other sibs.

20 AUG 1954 (38) - Litter of 6 to F.

22 AUG 1954 (40) 2 = M. This graft has a halo of tiny scabs at the graft/host skin junction, & may be having a mild reaction.

4 = F. Litter being well tended. Graft perfect.

24 AUG 1954 (42)
M. Small scabs - but no further serious deterioration.
F. A large ~~scab~~ posterior scab has suddenly appeared. The rest of graft is perfectly O.K. - could scab be non-specific?

26 AUG 1954 (44) M. Still some survival, but most of graft has scabbed.
F. Scab appears to be healing, and rest of graft appears to be healthy. Apply gammexane.

28 AUG 1954 (46) M. B.d. is probably just about complete.
Score: 46 days. Keep for 2nd stage graft.
F. High degree of survival with persistent hair-crop, but rest of graft is slightly inflamed; outlook not too good.

30 AUG 1954 (48) F. Still high degree of survival, but graft far from perfect.

~~31 AUG 1954~~

2 SEP 1954

F. Still high degree of survival: impression is that graft is recovering from severe reaction. New wave of pigmentation beginning? New hairs forming?

17 SEP 1954

(56) High degree of survival. Graft appears to have stabilised itself (though in a contracted form), but as yet little sign of new hair-growth. It is bald with the exception of a one or two small patches.

13 SEP 1954

Reoperation. (62)

M. True 2nd stage graft - 46 ± 16. Largish graft on LHS.
F. Animal pregnant again. Old graft still surviving, but only about 1/3 or more of original size. Dorsal scab, bald except for small tuft of cba hairs.
Graft largish graft on LHS.

Donor: cba Stock 22 female.

20 SEP 1954

7-day inspection of Male.
Epithelium peels with cuticle - trace survival. Note that healing and vascularisation are first-rate. Score: 8 days.
Kill.

Note that Female has had another litter.

24 SEP 1954 (62 ± 11)

Female: RHS (old): messy - probably little survival; trapped cba
LHS (new): breakdown far advanced - hairs.
about 25% survival.

25 SEP 1954 (62 ± 12)

Old graft - no change.

New graft - trace survival of small patch at graft centre-5%.

27 SEP 1954 (62 ± 14) LHS: patch of surviving ep. - now pigmented.

29 SEP 1954 (62 ± 16) Breakdown complete. *Destroy.*

Total score: 77 days.

Antigenic overloading effect.

Experiment concluded.

INOCULATION OF FOETAL MICE (CBA to A) WITH EMB-211
ADULT CELLS

14 MAY 1954 Donor: Exactly as for 210.

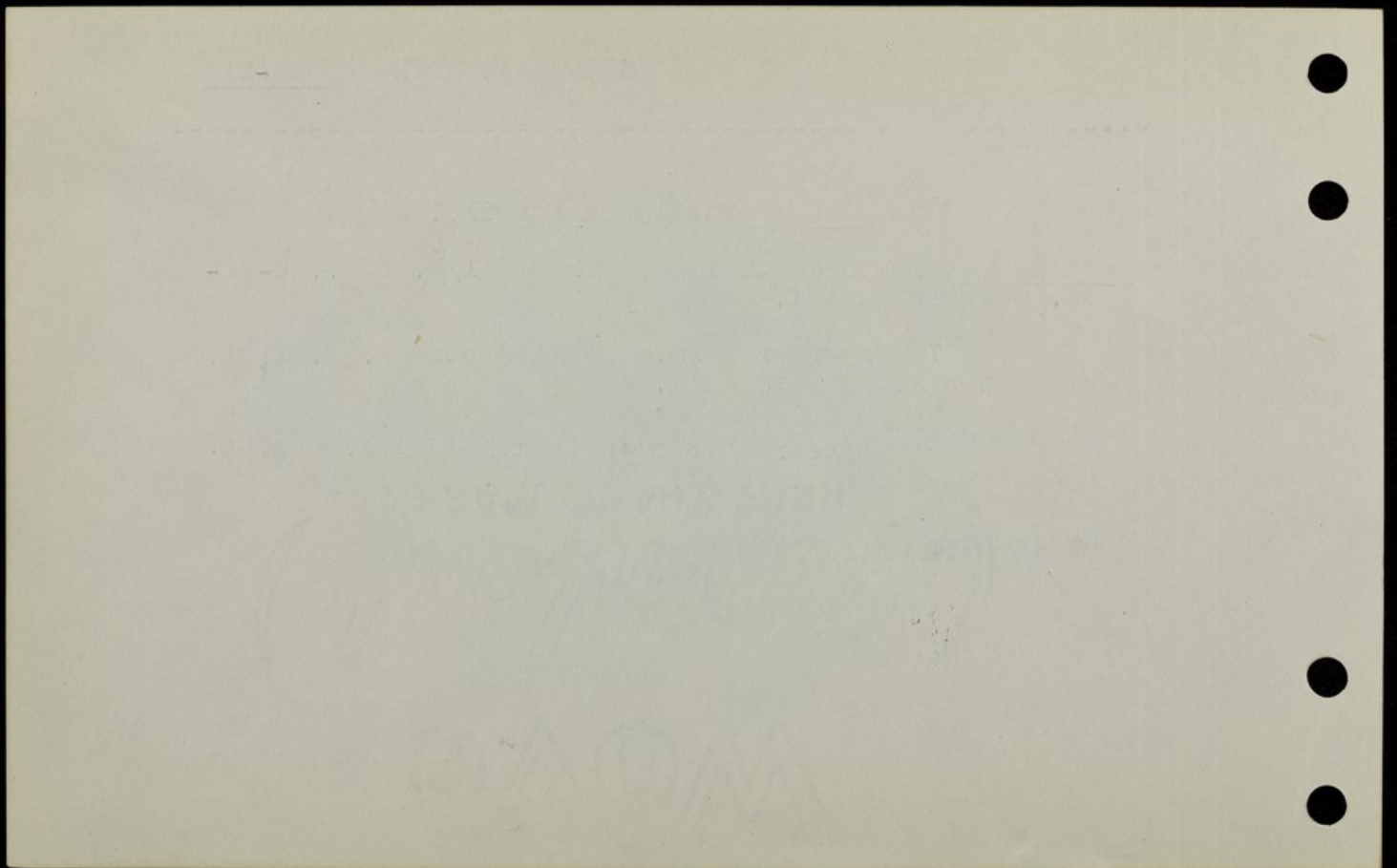
Recipients: Foetuses of female from 6A1/8. 37g., o-pa-
rous, G⁺entian violet.

7 very near term. Good injections. 0.01 ml.
Mouse not nesting very well.

15 MAY 1954 Litter born overnight. 4 alive, 3 dead.
Mouse has failed to nest. Unhopeful.

17 MAY 1954 All dead

EXPERIMENT CONCLUDED



INOCULATION OF FOETAL MICE (CBA to A) WITH
~~XXXXXXXXXX~~ ADULT CELLS

EMB 210

14 MAY 1954

Donor: Remove whole spleen, half kidney, small piece of liver, and one testis from Stock 15 cba male., chop up with scissors under normal saline in usual way, and produce the usual very dense suspension. Use coarse needle - plenty of cell-clumps.

Recipients: Foetuses of 10A7/P, representing 3rd litter. Wt. of mother: 47 g. Eosin.

9 foetuses - 17-18 days old. Visualisation good, and injections reliable. 0.01 ml.

16 MAY 1954 5 + 1 dead born: O.K. 17th.

16 JUN 1954 4 mice remain - 1 having died on the 11th June.

13 JUL 1954 Test-operation.
Donor: Stock ~~15~~ cba male Standard grafts and opns.

1 mouse unfortunately died post-op. Cause unknown.

23 JUL 1954 (10) 3/3: cuticles off fairly cleanly. Roofs somewhat delicate, but unquestionably 100% survival. Very little to choose between the 3. Rebandage on account of delicacy.

26 JUL 1954 (13) 2/3: b.d. complete. (1/2 with trace of pig.)
1/3: graft in very good condition, uncontracted, and lightly pigmented over whole surface. Super.

28 JUL 1954 (15) Slight contracture, some dorsal marginal scabbing, but rest of graft is pigmented and hair-growth has begun.

31 JUL 1954 (18) Graft is now doing fine. Scab has healed. No further contracture. Hair-growth in progress.

31 AUG 1954 (29) Hairs (dorsally directed) not quite as bushy as normal, and tiny imperfection of epithelium on ventral margin. Otherwise perfect. Run on.

16 AUG 1954 (34) Graft perfect.

24 AUG 1954 (42) Graft perfect.

30 AUG 1954 (48) Graft perfect.

1 SEP 1954 (63) " "

~~25~~ SEP 1954 (78) Graft perfect., except for tiny little superficial scabs on ventral margin that might be mite marks.

27 NOV 1954 (138) Considerable contracture has taken place as result of a nagging reaction, but about 1/2 (-) of graft remains and bears a respectable crop of hairs.

4 DEC 1954 (145) Graft now very small compared with original size: Dimensions: 11 mm x 3. But it is pigmented and bears a moderate crop of hairs. Clip.

9 DEC 1954 [150]. Inject I/P 0.5 ml = 25mg CBA lyophilized kidney suspension (see ANT-26). Note that the graft is slightly scabbed & definitely reactive, so that presumably the lyophilized injections will not work.

14 DEC 1954 (155) Graft has undergone definite deterioration since injection of lyophilised tissue. Severe dorsal scabbing has further reduced the area of survival.

31 DEC 1954 [172]. Long wispy hairs have growth from graft residue, which is about $\frac{1}{4}$ of original area. Survival nevertheless.

5 JAN 1955 [177]. Still further hair growth, though very wispy. There seems little doubt that foreign homologous cells survive.

SECOND STAGE GRAFTING. Donor: CBA 30 F.

13 JAN 1955 [177 + 8] LHS (new) graft 100% surviving. Old graft as before.

15 JAN 1955 [177 + 10] LHS (new) graft now only 75%.

SS61 NVF 21 [177 + 12] RHS graft is only a tiny strip with a few hairs. LHS now pretty well 100% (but contracted).

4 MAR 1955 (177 + 48) LHS (new): graft O.K. with reasonable crop of hairs. Still a little pink.
RHS: (old): graft a small remnant with a few hairs.

21 MAR 1955 (177 + 65) THIRD STAGE GRAFT.
Transplant another cba graft to high RHS. The other two grafts are as above.

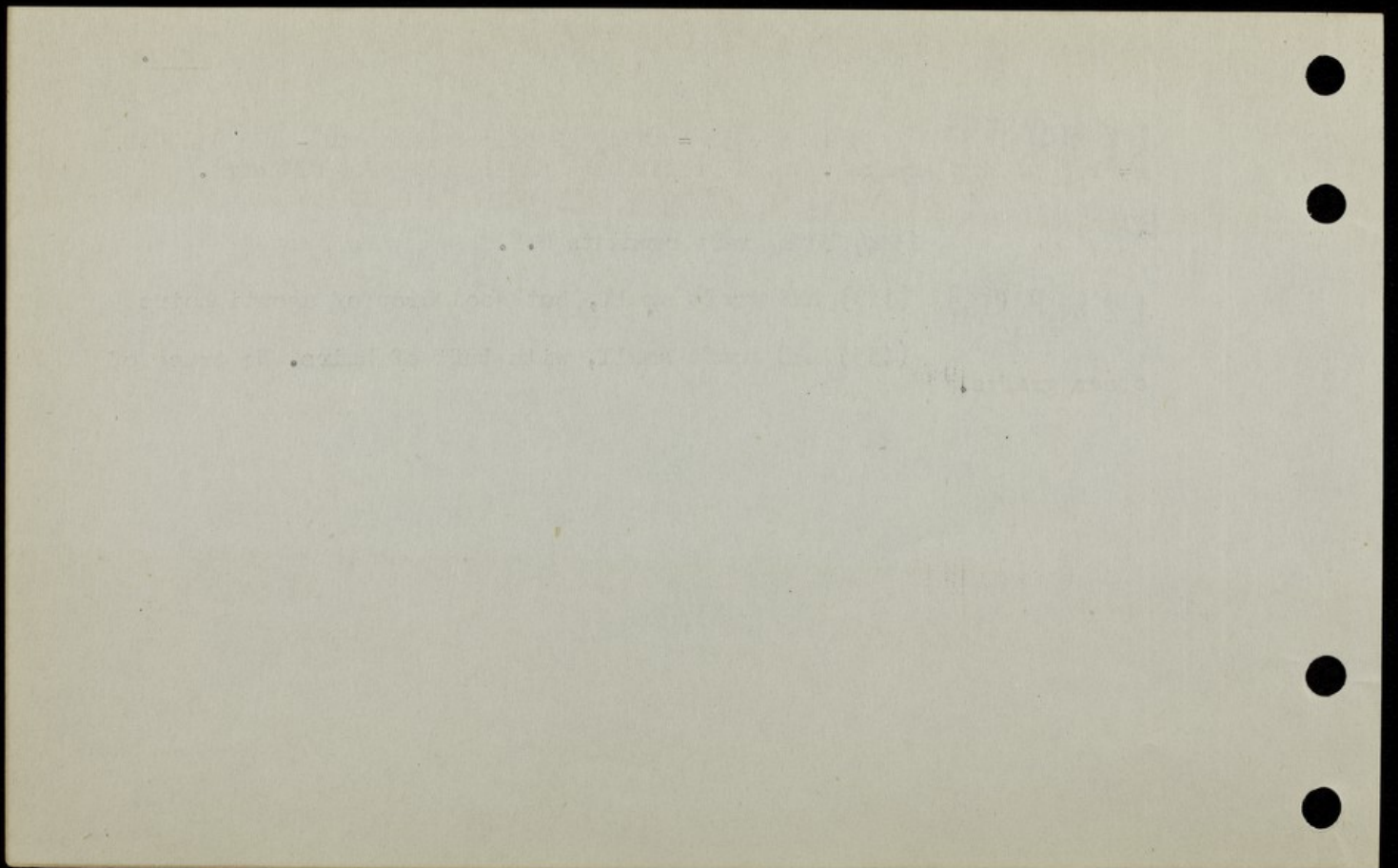
1 APR 1955 (177 + 65 + 11) Fresh graft in good shape - tolerance quite clearly continuing.

11 MAY 1955 (177 + 65 + 52 = 294) 2 haired remnants - RHS and LHS
(= 1st and 2nd grafts). Third graft (high RHS): good pelt of hairs.

1 JUL 1955 (344) Both graft remnants O.K.

12 SEP 1955 (418) LHS graft small, but good crop of agouti hairs

29 SEP 1955 (435) LHS graft small, with tuft of hairs. No trace of
other grafts.



INOCULATION OF FOETAL MICE (CBA TO A) WITH EMB-197
ADULT CELL SUSPENSION.

30 MAR 1954

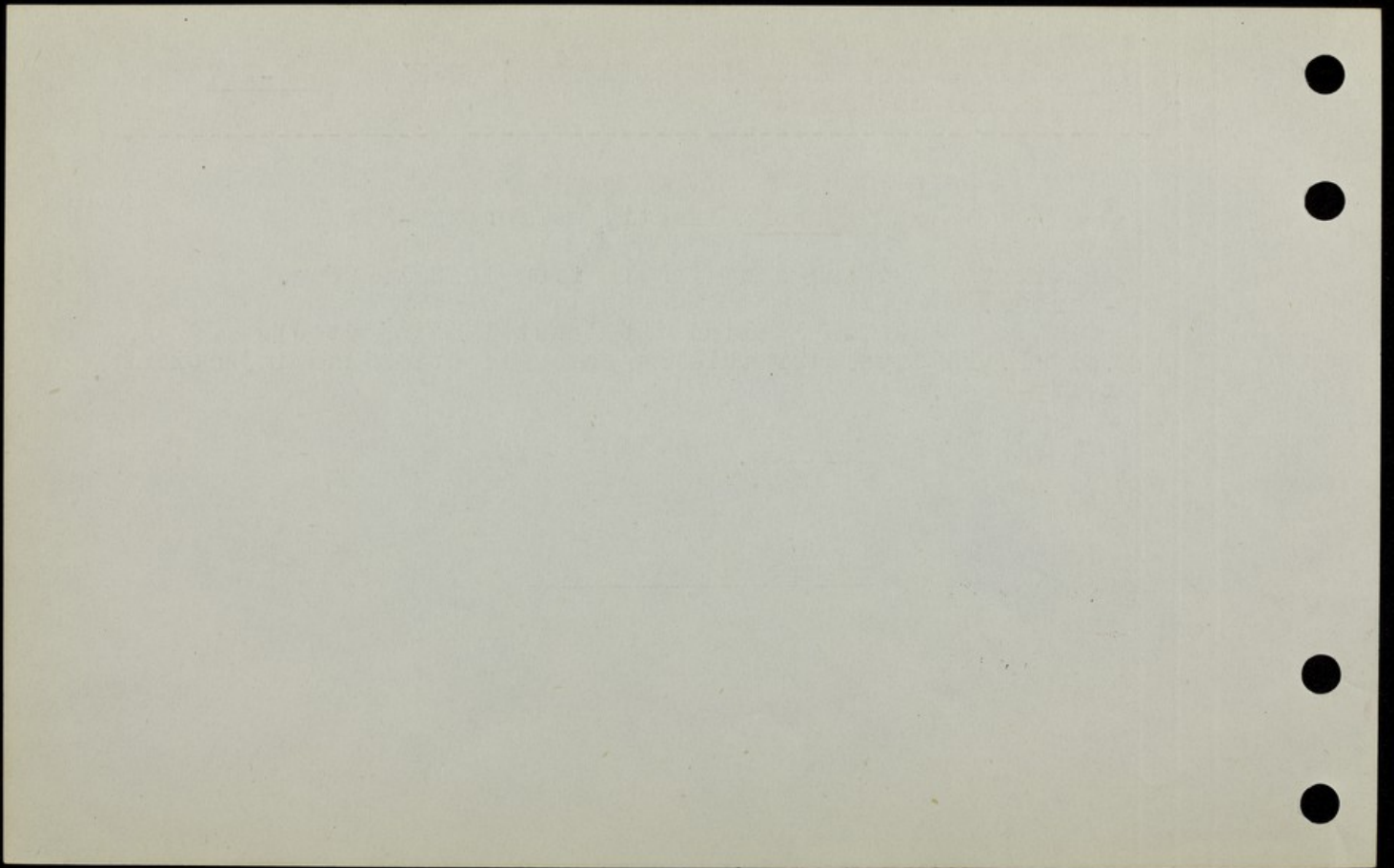
Donor: Exactly as for EMB-192.

Recipients: Foetuses of female from 10A2/1, 28 g.
0-parous.

5 plus 1 blind (at least 1 uninjected).
0.01 ml. 15 days. For this age visualisation and injections
pretty good.

3 APR 1954 Abortion.

Experiment concluded.



INOCULATION OF FOETAL MICE (CBA TO A) WITH
ADULT CELL SUSPENSION

EMB-196

30 MAR 1954

Donor: Exactly as for EMB-192.

Recipients: Foetuses of female from 8A5/3, 35 g.,
0-parous.

4 foetuses injected - very near term. 0.01
Visualisation and injections good.

2 APR 1954

Litter of 2. Age????

18 JUN 1954 TEST OPN. 2 male mice. Donor:- C XVI male.

28 JUN 1954 (10) Both show 100% survival, with some swelling. Mark R, O
& note that R-notch seems to be going.

29 JUN 1954

(11) R. Graft delicate, light central scabbing.
No. Graft very delicate and has small patches
of epithelial erosion, Prognosis poor.

30 JUN 1954 (12) R. Scabbing in progress, about 50% survival.
No. There appears to be little survival - graft covered with superficial scabs.

1 JUL 1954 (13) R. Dorsal 1/3 still surviving.
No. Graft appears to have recovered dramatically: scabs have disappeared, graft is in place, uncontracted, somewhat delicate, but almost fully epithelialised. Watch for hair-growth. (Overgrowth seems unlikely, but cannot be ruled out).

2 JUL 1954 (14) R. Survival now slight; trace.
No. Roof scurfy and a little scarred; some contracture; loss of pigmentation? Prognosis not good.

4 JUL 1954 (16) R. Breakdown just about complete. Score: 16 days.

(18) No. Still some survival.

12 JUL 1954 (26). No. Breakdown about complete. Score: 25 days.

Keep both mice for re-grafting.

22 JUL 1954 (36). 2nd stage grafting.
Donor: Stock 17 cba female. Standard LHS opns.

28 JUL 1954

(6) Healing fine in both.

R. Complete survival, but graft delicate. Rebandage.

No. Breakdown has just begun, but very high degree of survival. Rebandage.

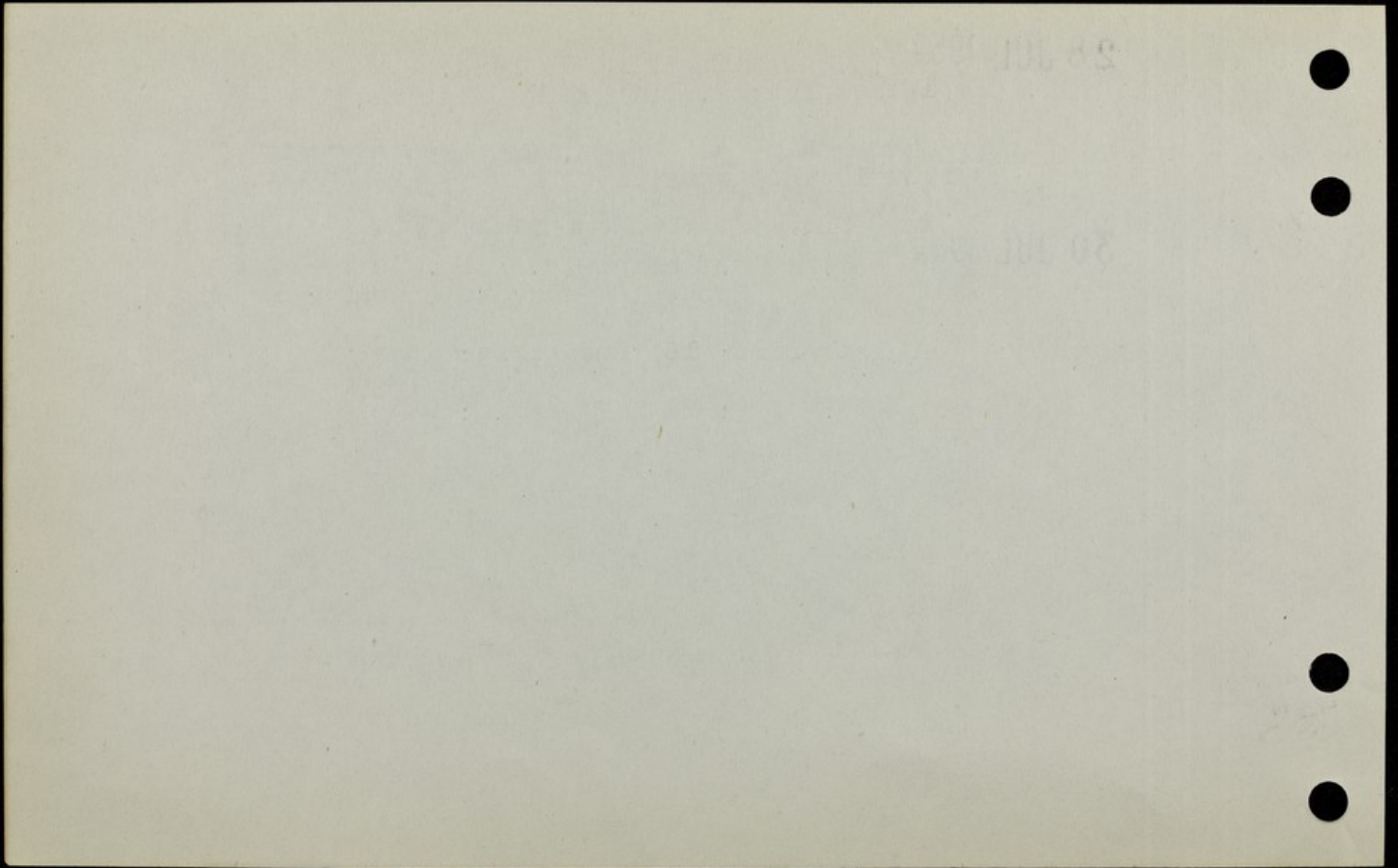
30 JUL 1954

(8) R. B. d complete. Kill. Score: 7 days.

No. About 10% survival. Score: 9 days.

Summary: R. 1st graft: 16 2nd graft: 7

No. " " : 25 " " : 9



INOCULATION OF FOETAL MICE (CBA TO A) WITH ADULT EMB-195
TISSUE SUSPENSION

30 MAR 1954

Donor: Exactly as for EMB-192.

Recipients: Foetuses of female from 7A5/7, 35 g., 0-pa-
rous.

8 embryos injected, age 17/18 days. 0.01 ml.
Hood visualisations and injections.

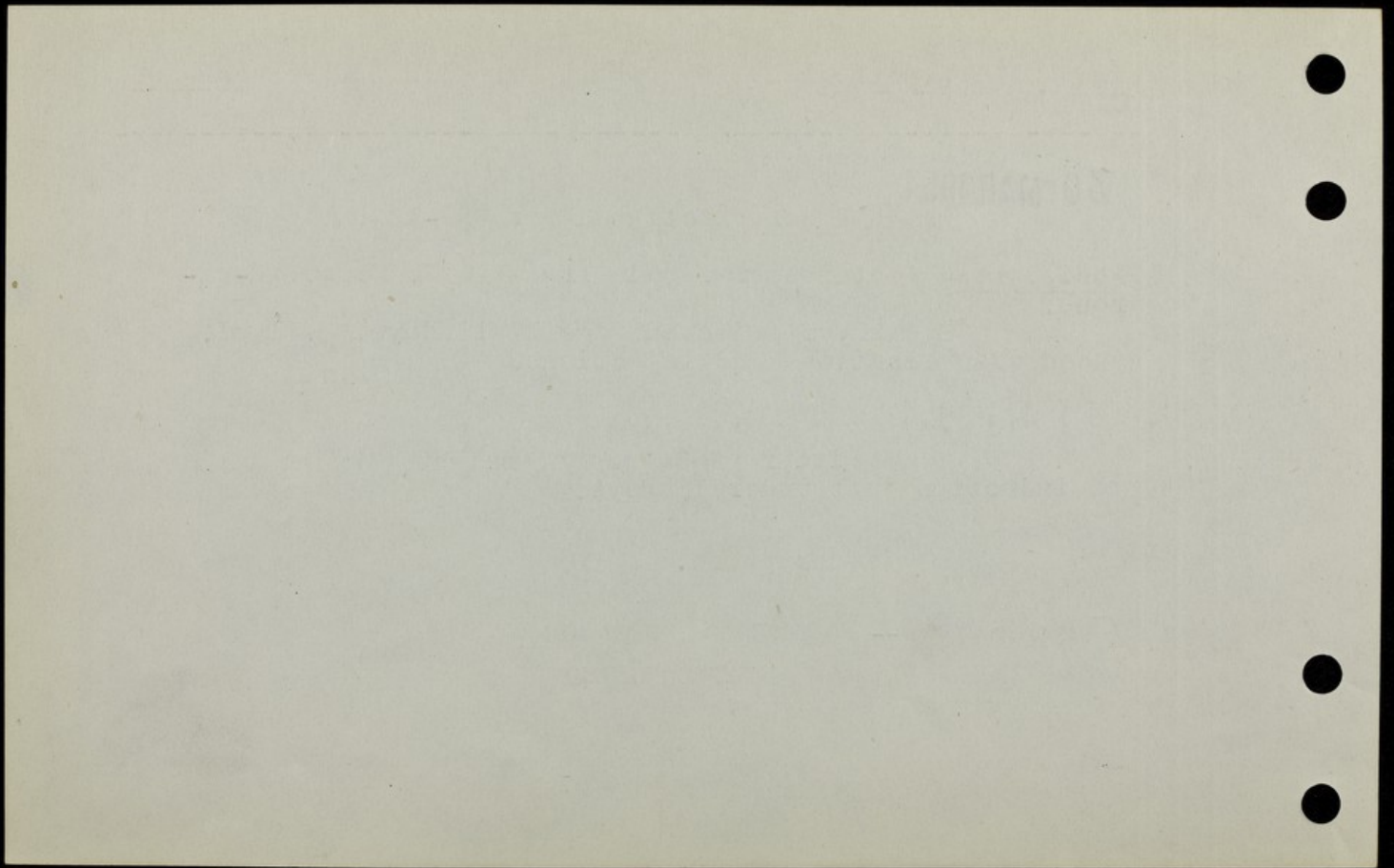
1 APR 1954

Litter of 3 born overnight. Age at time
of injection just under 18 days.

2 APR 1954 2 survivors.

[18 JUN 1954 -- NO TRACE OF THESE MICE]

EXP CONCLUDED



INOCULATION OF FETAL MICE (CBA TO A) WITH ADULT EMB-194
TISSUE SUSPENSION

30 MAR 1954 Donor: Exactly as for EMB-192.

Recipients: Foetuses of female from 8A7/2, 32 g., 1-pa-
rous.

Inject 4 - 3 definitely, 1 less so. Either a 5th
was injected, or else one of the 4 twice. 0.01 ml.
Age very near term. Visual. quite good.

31 MAR 1954 Litter born this afternoon, about 4 p.m.
2 alive, 1 dying, and others mauled.
The live ones look healthy enough.

2 APR 1954 The 2 survivors are doing well.

18 JUN 1954 TEST OPERATION. 2 mice. Donor:- C XIV male

28 JUN 1954 (10) Both show 100% survival. Mark O and R.

29 JUN 1954 (11) R. Breakdown complete. Destroy.

No. 100% survival, but graft delicate with points of epithelial erosion.

30 JUN 1954 (12) No. Scabbing in progress - about 50% survival.

1 JUL 1954 (13) No. Less than 50% survival, but the surviving patch looks fairly healthy and is lightly pigmented.

2 JUL 1954 (14) Anterior 1/4 looking very healthy; rest has scabbed.

6 JUL 1954 (18) Patch still holding out; surface generally bald, but a few pigmented hairs can just be distinguished.

22 JUL 1954 (24) Just over half of the original graft is still surviving. Bald, but there are a few pigmented hairs.

22 JUL 1954 (34) Pigmentation has intensified, and graft remnant is now sprouting cba hairs. Animal is clearly tolerant and will soon have to be regarded as SUPER. This is a remarkable recovery.

31 JUL 1954 (43) Graft remnant has grown reasonable crop of hairs. Mouse should soon receive 2nd graft.

11 AUG 1954 (54) Graft remnant still carries thin wispy crop of agouti hairs. **REGRAFT.**

12 AUG 1954 (55) Regrafting. Donor: CBA XVI F. **LHS opn.**

Temporary mark: Eo head Skin graft active. Wt. of mouse 28g.

19 AUG 1954 (55 + 7). Second stage graft. The **RHS** (first-stage) grafts represented by two separate pigmented patches.

LHS graft well healed. Ventral half **active** and shows some almost certainly non-specific superficial breakdown. From dorsal half, note clean separation of cuticle, 100% epithelial survival, though graft rather dark pink in colour and with delicate appearance.

21 AUG 1954 (55 + 9). **RHS** graft as before.

LHS (2nd-stage) graft:- The active ventral half noted 2 days ago has formed a healthy dry crust (non-specific breakdown). Rest of graft (50%+) has firm epithelium, dark smoky-pink graft: **100%** survival.



23 AUG 1954 (55 + 11). **RHS** graft dark, new hair growth probably beginning. **LHS** (2nd-stage);- Dorsal $\frac{1}{2}$ (see above) O.K., pigmented. Scab off ventral half to reveal **smoky pink surface**, 100% survival but somewhat delicate.

24 AUG 1954 (55 ± 12)

RHS (old): graft undergoing scabbing. Probably still some survival.
LHS (new): b.d. just about complete.

26 AUG 1954 (55 ± 14)

RHS: there may be very small graft remnant.

LHS: it is just conceivable that there is some graft survival -
but overgrowth of the graft dermis by native epithelium is
not unlikely.

28 AUG 1954 (55 ± 16) Survival of homologous tissue in either of the
grafts is now extremely unlikely. Since the 2nd graft was not a true
2nd stage graft, score combined survival as 71 days.

Keep for genuine 2nd stage graft.

2 SEP 1954

(55 ± 21) Mouse pregnant. Note that 'scars' on both sides
may be pigmenting - there may therefore be continued survival.

WATCH.

7 SEP 1954

(55 ± 26) Litter born. Remnant grafts on both sides have
developed pigment and are growing some cba hairs. Tolerance clearly
still in force. Keep for 3rd graft.

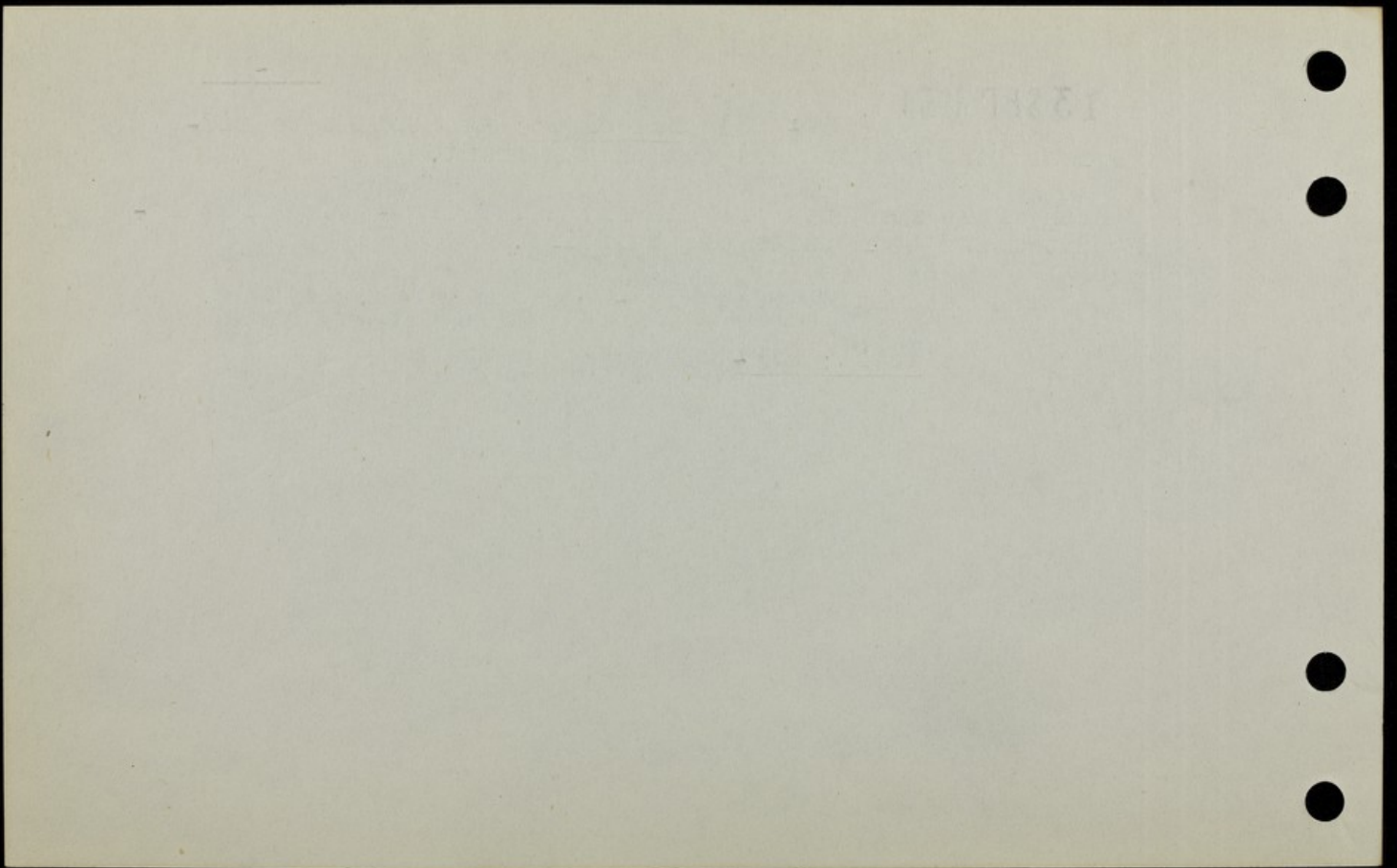
EMB-194

13 SEP 1954 (55 ± 32) 3rd graft: transplant to mid-dorsal line a third cba (stock 22) graft.

Both graft remnants are clearly surviving - they are pigmented and bear tufts of cba hairs.

Mouse dead post-operatively - cause unknown.

Score: 87 ±



INOCULATION OF FOETAL MICE (CBA TO A) WITH ADULT
TISSUE SUSPENSION

EMB-193

30 MAR 1954

Donor: Exactly as for EMB-192.

Recipients: Foetuses of female from 8A3/3, 28 g. 0-pa-
rous.

Inject 4 definitely, but at least 1 not at all
injected.

Age 17-18 days. 0.01 ml. (Plus in some).

Mouse difficult - ether and some rather rough handling.

2 APR 1954

Litter of 3. Age at time of injection therefore
17 days.

18 JUN 1954 TEST OPERATION. 3 mice. Donor: C XVI male.

28 JUN 1954 (10) All three = 100%: mark L,R,O. One of these is swollen
and rather dark pink (viz: no-notch).

29 JUN 1954

(11) R. Nearly 100% survival, but small dor-
sal patch of scabbing.

L. 100% survival - very light pigmentation.

But very small dorsal patch of epithelial erosion.

No. Nearly 100% survival - but graft delicate and with some points of epithelial erosion.

30 JUN 1954 (12) R. About 50% survival.
L. 100% survival, but graft rather delicate. Slight pigmentation.
No. Just under 50% survival - scabbing in progress.

1 JUL 1954 (13)
R. Still about 50% survival.
L. Much as yesterday.
No. About 25% survival. The surviving patch looks pretty healthy and is lightly pigmented.

2 JUL 1954 (14) R. Survival still about 50%. The epithelium is lightly pigmented, and impression is that the scabbed areas are healing.
L. Still complete survival; pigmentation very light and patchy; a few hairs have penetrated.
No. There appears to be a high degree of survival, probably because the scabbed areas have been shed, leaving the healthy part. There is some pigmentation, and some hairs have just penetrated.

2.

6 JUL 1954 (18)

EMB-193

- R. Graft recovering - graft remnant (rather less than 50% of original graft) is now growing cba hairs.
- L. Graft is O.K. except for small dorsal and marginal scab which refuses to heal. Pigmentation still scanty and graft generally bald - a few isolated longish cba hairs.
- No. Graft has recovered remarkably well. There is now extensive hair-growth over the remaining area - representing about $\frac{1}{2}$ of the original graft.

12 JUL 1954 (24)

- R. Recovery has progressed slowly but steadily. Graft remnant has a thin crop of cba hairs.
- L. Dorsal and ventral scabbing has taken place over last few days, but central area representing more than $\frac{1}{2}$ of graft is still surviving; it is bald except for a few long cba hairs.
- No. Recovery is now complete: graft has good crop of cba hair and is beautifully supple.

22 JUL 1954 (34)

- R. Graft remnant has recovered completely, and is now bearing quite a bold patch of cba hairs. Mouse must now be regarded as SUPER.
- L. Rather less than $\frac{1}{2}$ of original graft still surviving, but almost completely bald. A few wispy pig. hairs have been present for some time - more hopeful sign is a faintly pigmented patch. Graft has suffered quite clearly from a severe reaction.
- No. Graft in excellent form. Undoubtedly SUPER.

31 JUL 1954 (43)

- R. Graft remnant doing fine - good crop of hairs. Animal should soon receive 2nd graft.
- L. Still no hairs; contracture of graft remnant still further advanced, but still some survival. Faintest trace of pig. Good example of borderline tolerance; keep for 2nd stage graft when b.d. is complete.
- No. Graft in excellent form.

11 AUG 1954 (54)

- R. Hairs wispy, but about $\frac{2}{3}$ graft well defined. REGRAFT.
- L. No sure evidence of survival: merely scar. REGRAFT.
- No. Graft perfect. READY FOR NODE INOCULATION.

12 AUG 1954 (55) Regrafting of R & L. Donor: CBA XVI F. LHS OPERATIONS.

Note:- L has temporary colour mark (Gen head); R has only ear mrk.

R. 20g. Graft of normal skin

L. 27g. Skin graft active.

16 AUG 1954 (60) Inoculation of 'No ear mark' with normal node fragments

Procedure as under EMB-192, except that the usual PT method of node inoculation was used. Most unfortunately this mouse died of haemorrhage from pucture of IVC: see notes to EMB-192 and follow that procedure in future. Note weight of this mouse only 21½ g.

While still alive **PHOTOGRAPH IN COLOUR**. Graft not quite perfect (contracted AP and with a tiny epithelial blemish) but not the least reason to think it would have been anything but a permanent survivor). **TAKE SPECIMEN** (with REB).]

SCORE: 80
60++

19 AUG 1954 (55 + 7) SECOND STAGE GRAFTS.

R. (This mouse only 18 g). Healing sound. Full separation of cuticle. 100% epithelial survival, though graft is rather dark pink and delicate-looking. Smear vaseline on RHS graft & replaster.

L. (temp. mark: gentian head). There has been a slight downwards

displacement of graft on bed, and ventral half is highly active. Disregarding ventral overlap, dorsal 2/3rds OK. Note clean separation of cuticle, epidermis 100% surviving though colour dark smoky pink (incipient activity). Rather delicate looking. Nevertheless CLEAR DEMONSTRATION OF WEAKENED SECOND STAGE RESPONSE (survival time of 1st-stage graft can be taken as 48 days, half-way between 43 & 54).

Replaster.

21 AUG 1954 (55 + 9)

- R. RHS graft OK. LHS (2nd-stager) still has delicate appearance, and cuticle is flat & crackled. But 100% survival certain.
- L (gen head) Much as EMB-194. Ventral half (active zone) has formed a crust. Rest is dark smoky pink with 100% ep survival.



23 AUG 1954 (55 + 11)

- R. RHS graft OK. LHS (2nd-stage) 100% survival, still with remains of 'marbled' appearance (may be partly due to soaking up of thick vaseline by cuticle).
- L (gen head) Dorsal half delicate, but still a shaded 100% survival: will break down (already at MST + 1 for normal grafts). Scab off ventral half: not quite completely epithelialized: ingrowth.

24 AUG 1954 (55 ± 12)

R. RHS (old): some scabbing of graft remnant taking place.
LHS (new): 100% survival - graft rather delicate and a little puffy, but light pigmentation and a few wispy hairs.

L. B.d. just complete. SCORE: 12 days. Kill.

26 AUG 1954 (55 ± 14)

R. RHS: graft survival very doubtful but just possible.
LHS: further contracture, otherwise as above. No hairs.

28 AUG 1954 (55 ± 16) LHS graft has not deteriorated further. 100% survival.

30 AUG 1954 (55 ± 18) LHS graft is O.K. and is now growing several patches of hairs. Tolerance is clearly continuing.

2 SEP 1954 (55 ± 21) LHS graft has now distinguished itself by pretty good hair-growth. RHS graft remnant is also clearly surviving, for it is re-pigmenting.

7 SEP 1954 (55 ± 26) RHS: remnant has pigmented and is growing some cba hairs.

LHS: graft is healthy and growing

good crop of hairs - some of these are white - possibly due to earlier nagging reaction.

18 SEP 1954 (55 ± 37) LHS graft is O.K. and bearing quite a good tuft of hairs. RHS graft remnant bears tiny tuft.

22 SEP 1954 (55 ± 41) O.K.

29 SEP 1954 (55 ± 48) Ditto.

5 OCT 1954 (55 + 54 = 109) ADOPTIVE IMMUNIZATION (IMMUNE NODES)

Take 7 prs = 14 hypertrophied homolateral axillary nodes from 7 A-line mice at MST + 1 (CBA on A) of series ANT-18, choosing 7 mice in which breakdown of the CBA graft was complete.

Prepare expressed node cell suspension by sieve method in normal citrate-saline; total, about $1\frac{1}{2}$ ml. Intraperitoneal.

8 OCT 1954 (109 + 3) There has been some hair growth since 109 in the crescent-shaped zone of the graft that was 'active'. Today: graft is definitely palpable and swollen, with hint of dark pink to livid colour.

9 OCT 1954 (100 + 4). LHS graft now shows depressed central scab, which had begun to form at $3\frac{1}{2}$ days and is now further developed.

(EMB-193)

~~10~~ **10 OCT 1954** (109 + 5). Further deterioration. More contracture; scab extends to anterior margin; rest of epidermis is very weak and hairs are obviously only trapped in like-like position.

11 OCT 1954 (109 + 6). Breakdown can now be taken as complete in both grafts, with generalized scabbing. Note that the reaction was of remarkable speed & violence. This mouse in poor shape.

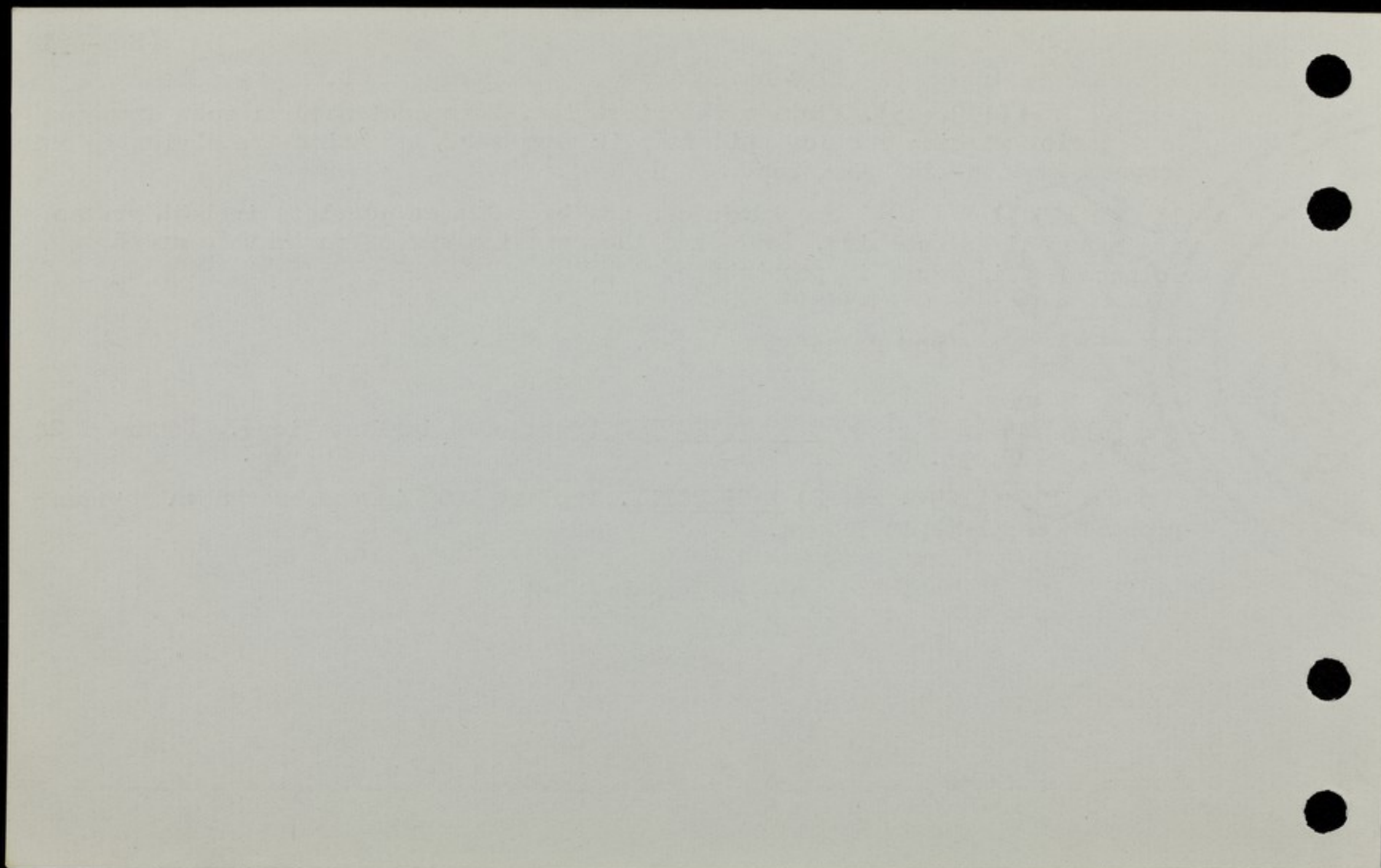
~~OCT 1954~~

For regrafting later, with EMB-192

29 OCT 1954 (109 + 24) TEST OPERATION (post node implantation). Donor: C 24 female. RHS opn. See also EMB-192.

6 NOV 1954 (109 + 24 +8) INSPECTION. Typical 100% immune breakdown. B'down probably complete at 7 days.

EXPERIMENT CONCLUDED



INOCULATION OF FOETAL MICE (CBA to A) WITH ADULT
TISSUE SUSPENSION

EMB-192

30 MAR 1954

Donor: Kill 7C6/P male and remove both testes, one kidney, spleen and small piece of liver. Chop up in usual way to produce dense cell-suspension. Use fine needle.

Recipients: Foetuses of female from 8A7/2, 31 g. 0-pa-rous.

Inject definitely 4, 1 almost certainly, and a 6th was less certain. 2 of those well injected developed internal haemorrhages.

Age about 17 days. 0.01 ml. Good visualisation.

22 APR 1954

Litter of 3 born overnight. Age at time of injection therefore 17 days.

18 JUN 1954 T E S T O P N. 2 mice. Donor:- C XVI male

28 JUN 1954 (10) One = 0%. Destroy.
One = 100% but fault of healing D/P: rebandage. Note ventral half has pigment shadow.

29 JUN 1954 (11) Graft with 100% survival, looks healthy and has leaden grey pigmentation. Supermouse?? Dorsal fault has healed almost completely.

1 JUL 1954 (13) Graft with incipient hairgrowth.

2 JUL 1954 (14) Graft undoubtedly super. Vigorous hair-growth over whole surface.

6 JUL 1954 (18) Graft doing fine. Very dense hair-growth - graft a little palpable but this probably due to intense follicular activity.

12 JUL 1954 (24) Graft is O.K. - pelt of hairs very dense. But there is still a small marginal scab which robs the graft of full suppleness. Scab could be due to very feeble reaction - though rest of graft gives no such indication. In case it has been caused by mites (and scratching), dust with gammexane powder.

22 JUL 1954 (34) Graft in very good shape. Now completely supple and without scabs. Undoubtedly SUPER. Note that animal is somewhat delicate.

31 JUL 1954 (43) Graft in good shape.

1954
11 AUG 1954 (54) Graft perfect. READY FOR NODE INOCULATION.

16 AUG 1954 (60) INOCULATION WITH NORMAL NODE TISSUE. Take 2 inguinal, 4 axillary, 4 cervical nodes from two A-line Stock 19 males: total 20 nodes. Chop up in usual way and load into mounted trochar.

PHOTOGRAPH IN COLOUR the mouse (a) with hairs (b) clipped (c) shaved.

Then tie out as for abdominal operation. Make $1\frac{1}{2}$ cm ventral midline incision in skin of abdominal wall somewhat posterior to midline. Then make a tiny transverse snick in the abdominal wall and pour in the contents of the loaded trochar-pipette. Close abdominal wall with 2 sutures & skin with two sutures. Allow to recover in incubator.

Note weight of mouse only 19g, but perfectly lively.

NOTE ON PROCEDURE: In view of disaster with EMB-193 (photographed in parallel) adopt the above procedure in future, exc. introducing the node fragments with an ordinary widish bore glass pipette.

The graft is not quite perfect cosmetically, owing to AP contracture & a minute superficial plaque-like scab dorsally. Area

4~~5~~ mmAP x 8 mm DV

19 AUG 1954 (160 + 3). Graft has been watched over past 3 days very carefully. The tiny dorsal plaque noted at 60 days has spread a tiny bit, and is now a micro-scab covered with dried serous exudate. There is also now a tiny dorsal scab. Rest of the graft looks perfectly sound.

IN VIEW OF THESE IMPERFECTIONS - hinting at incipient or low grade reaction quite apart from node inoculation - THIS MOUSE PROBABLY NOT SUITABLE FOR SERIAL PHOTOGRAPHY.

21 AUG 1954 (160 + 5). The little eczematous plaques have spread still further, but rest of graft has active appearance and new hair growth has just begun.

23 AUG 1954 (160 + 7). Graft has improved; further hair growth, longest hairs being 2-3 mm long, and rest of graft dark & active-looking. Further there has been a slight contracture associated with the adequate healing of the scar-scabs noted earlier. Thus no question of any new immunity reaction's having begun.

24 AUG 1954 (160 + 8) Scabs seem to be healing. Vigorous hair-growth in ventral half.

26 AUG 1954 (60 + 10) Scabs have healed; graft supple and without further signs of deterioration. Hair-growth over whole of surface. CLIP

28 AUG 1954 (62 ± 12) Hair-growth is still vigorously taking place - new hair-crop has been grown in last 2 days. Graft is now completely healthy: no sign of reaction.

30 AUG 1954 (62 ± 14) Small marginal scabs anteriorly; rest of graft healthy and growing hairs vigorously.

31 AUG 1954 (62 ± 15) Marginal scabs persist, but rest of graft still healthy, deeply pigmented and with hair-growth. But note that the graft has undergone considerable contracture during the last few days: in view of the graft's recent history this is likely to be due to a chronic spontaneous reaction - not to the implanted nodes.

2 SEP 1954 (62 ± 17) The graft remnant is healthy - still no sign of a generalised reaction.

7 SEP 1954 (62 ± 22) Clip graft and examine: graft now small but healthy.

13 SEP 1954 (62 ± 28) Graft is slightly inflamed and swollen, and marginal scabs have appeared. Beginning of specific reaction???

18 SEP 1954 (62 + 33) Scabbing of graft has continued; but there is still a patch which looks healthy and may even be carrying a tuft of growing hairs. This latter observation is disquieting.

20 SEP 1954 (62 + 35) Small healthy-looking patch persists; clip tuft of hairs.

23 SEP 1954 (62 + 37) BREAKDOWN OF GRAFT IS PROBABLY JUST ABOUT COMPLETE. There is still a tiny spot bearing a tuft of hairs, but but these are almost certainly trapped in the scabbed graft dermis. Confirm b.d. to-morrow.

25 SEP 1954 (62 + 39) Breakdown appears to be complete.

29 OCT 1954 (62 + 39 + 34) TEST GRAFT (Pst implantation of nodes). RHS opn; C 24 female donor.

6 NOV 1954 (62 + 39 + 34 + 8) Inspection.

Graft shows 25% survival clearly; rest of epidermis (though thick, and cuticle peeled off cleanly) comes away from dermis. Survival time can be taken as 9 days, i.e. weak persistent immunity after node inoculation.

EXPERIMENT CONCLUDED

24 MAR 1954 DONOR: Exactly as EMB-189.

RECIPIENT:- 8A1/P female of breeding pair, now in course of 6th pregnancy. 53 g. [See EMB-166]

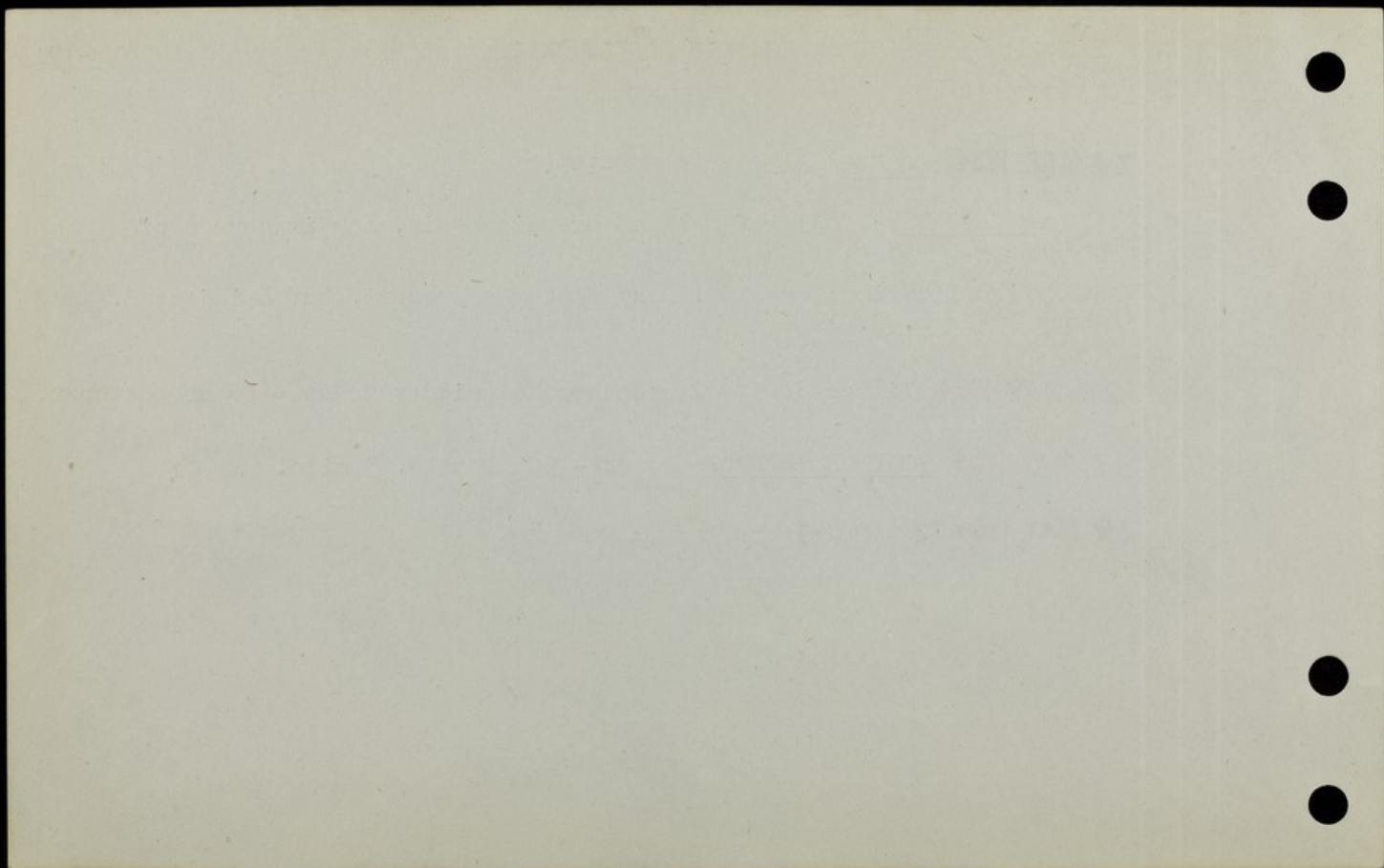
Visualization very bad: many fetuses present, but 6 injected. Age about 18 days. 0.01 ml. **Wide bore needle.**

26 MAR 1954 Litter of 5 + 2 dead born at midday today = 48 hr postoper.

17 MAY 1954 TEST OPERATION. Donor:- 9B1/1 male. 3 mice.

29 MAY 1954 (12) Total breakdown: destroy.

EXPERIMENT CONCLUDED



24 MAR 1954 DONOR:- As EMB-189 q.v.

RECIPIENT:- 8C3/2 female in (?) 2nd pregnancy by litter mate.
40g.

Inject 0.01 ml into 5 18-day fetuses. One injection uncertain, and a sixth foetus seen but not injected. **Wide bore needle**, horizontal approach.

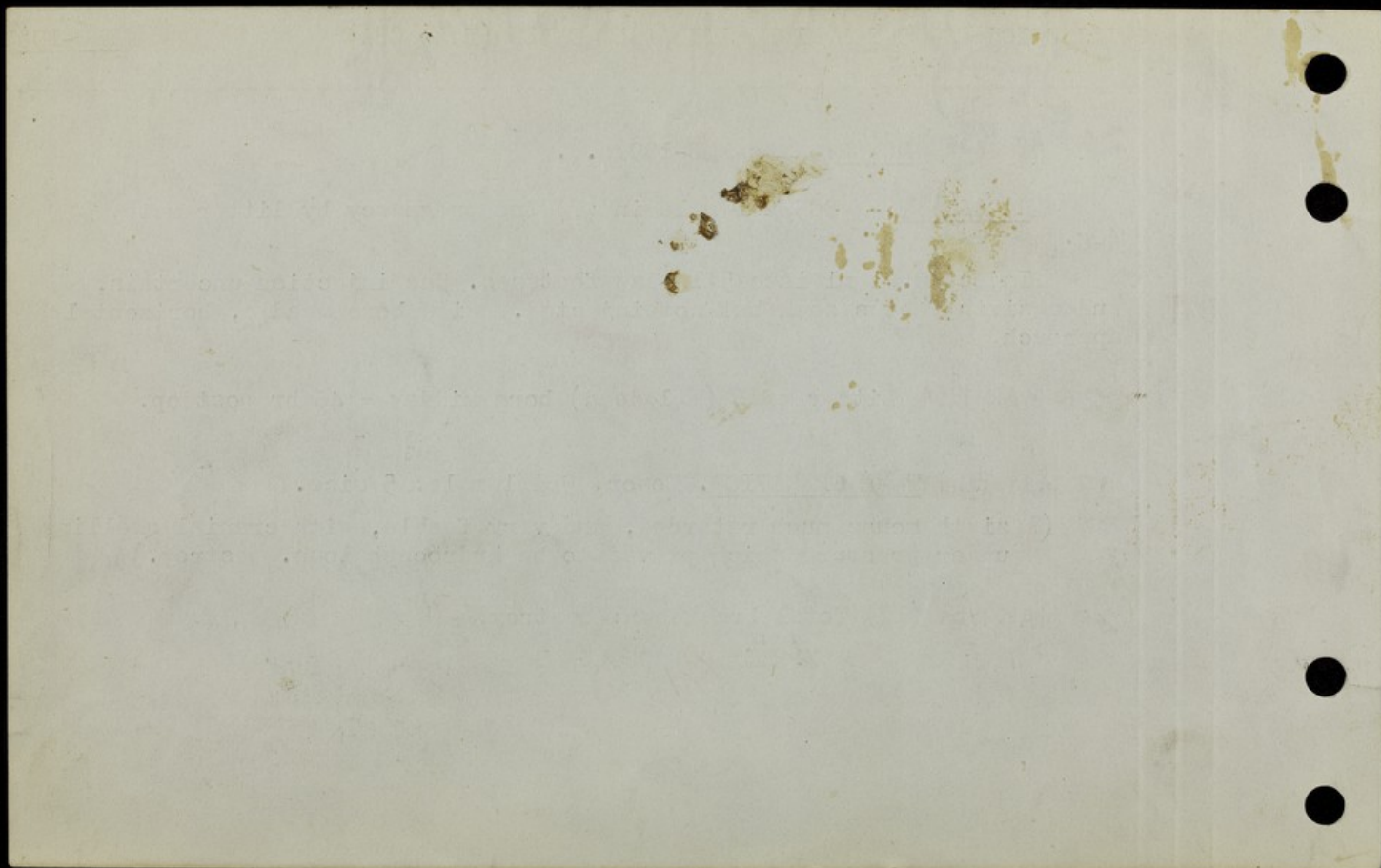
26 MAR 1954 Litter of 6 (+ 1 dead) born midday = 48 hr post op.

17 MAY 1954 TEST OPERATION. Donor: 9B1/1 male. 5 mice.

(A sixth mouse much retarded, and very feeble, with cranial swelling under pressure which proved to be hydrocephalous. Destroy.)

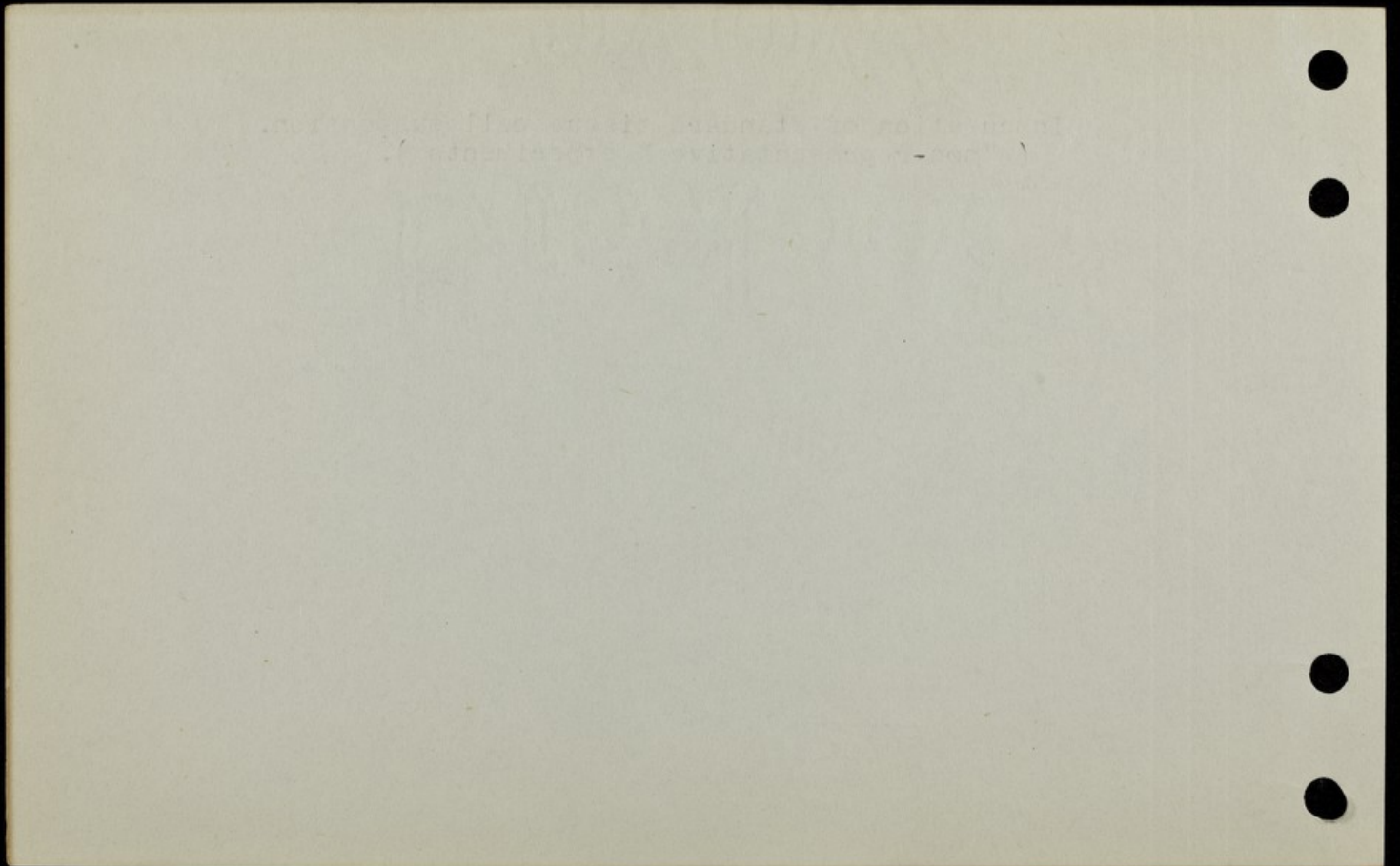
29 MAY 1954 (12) Total breakdown: destroy.

EXPERIMENT CONCLUDED



C.

Inoculation of standard tissue cell suspension.
("non-representative " experiments).



INTAEMBRYONIC INOCULATION OF FOETAL MICE (A to cba)
WITH ADULT CELL SUSPENSION THROUGH BODY WALL.

EMB-79

16 FEB 1953 Donor tissue: Kill male of litter 4A3/3, b.6 Jan., and remove one testis, whole spleen, a small piece of liver, and a 1/4 kidney. Chop up with scissors and prepare a very dense suspension (brei) of cells. Inject each foetus with 0.01 cc.

Recipients: Foetuses of female of litter 3C4/3, b. 8 Nov., pregnant by litter-mate, 32 g., supplementary diet since 17 Dec.

6 foetuses seen and injected intraembryonically. Age 15-16 days. Visualisation fairly good but size of foetuses a slight handicap. Injections fairly satisfactory - one rather blind. 0.01 cc. each.

19 FEB 1953 Litter of 3. Remnants of two newborns found. Birth probably took place in early hours of morning - age at time of injection therefore about 16 days. Survivors doing quite well.

21 FEB 1953 Only 2 survivors remain. 2

14 APR 1953 Test operation.

Donor: Remove standard body-skin pinch grafts from female of litter 4A2/3, b.12 Jan., scrape off panniculus, and transplant to scissor-clipped bed in usual way.

1. F 19 g. Opns. satisfactory.
2. M 21 g.

22 APR 1953 (8)

Female. Healing faultless. Posterior margin somewhat inflamed. Cuticle clings tenaciously and cannot be pulled off from centre which is very weak. Prognosis poor. Rebandage.

Male. Healing faultless. Tulle off cleanly. Total survival but two patches of superficial haemorrhage are indicative of incipient reaction. Rebandage,

25 APR 1953 (11)

Female. Graft of good colour with firm epidermis except for central healing ulcer patch corresponding to adherent cuticle of 8 day report. Could be just fault of healing. ~~Rebandage~~ Germolene.

Male. Typival breakdown. Kill.

27 APR 1953 (13)

Female. Roof dry, firm, and white - obviously epithelialised, covered by scaly cuticle. Central scar seems to be healing. Graft is thick and probably also swollen and stands out in sharp relief against the thin host skin. A mild reaction may be taking place but graft is holding its own.

28 APR 1953 (14) Contraction has taken place, and the central scar has now merged with another marginal one. A horse-shoe of white graft tissue, covered by epithelium, is still present, but graft is on the way out and is not likely to live more than another day or two. Graft still very thick.

29 APR 1953 (15) Graft now red and about to scab.

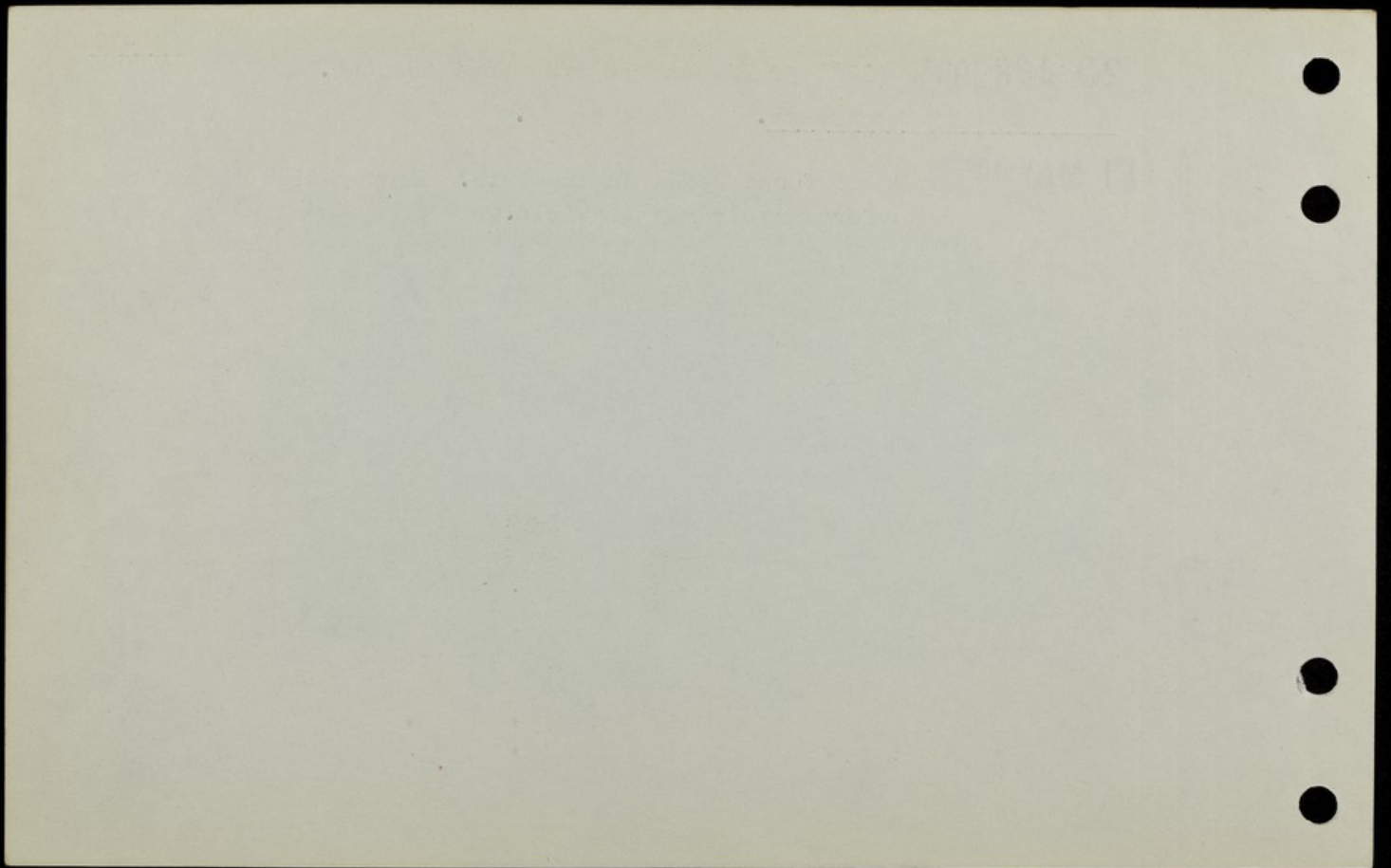
Breakdown just complete.

Reoperate.

11 MAY 1953 Mouse found dead on the 10th. Reoperation therefore
unfortunately not possible.

11 1953

11 1953



INJECTION OF FOETAL MICE (AU to CBA) WITH MIXED ADULT
WHOLE BLOOD AND CELLS FROM SPLEEN & NODE

EMB-162

16 JAN 1954 INJECTION MEDIUM:- Exactly as EMB-159 q.v.

RECIP:- 8C3/2 female (16 Nov) 30g. Pregnant by litter mate. See 4 + 1
15-day approx foetuses, injecting 0.005 ml. Visualization of one horn good
& injections surprisingly satisfactory for this age.

21 JAN 1954 Healthy litter of 5 born overnight - i.e. $4\frac{1}{2}$ days after
injection.

23 JAN 1954 One found dead; the other 4 a bit feeble but look as if they
might pull through.

26 MAR 1954 TEST OPERATION. Donor:- 8B1/3 male.

5 APR 1954 (10) 2/4 with advanced or completed breakdown. Kill.

2/4 with very high degree of survival, but one is definitely weak
and the other has some haemorrhagic spotting. Both deteriorated fairly
rapidly on drying. Prognosis very bleak.

6 APR 1954 (11) 1/2 with breakdown just complete. Kill.
1/2 with high degree of survival but severe contracture
and some discolouration.

- L 7 APR 1954 (12) Still high degree of survival.
- L 8 APR 1954 (13) Some survival, but graft deteriorating.
- 14 APR 1954 (19) Graft has contracted steadily and is now a small bald, shiny patch - but donor epitheliumis probably still hanging on.
- 16 APR 1954 (21) Contracture ans scabbing have continued, still bright survival. Scire as breakdown to-morrow.

Score: 22 days. Keep for 2nd grafting.

7 MAY 1954 (42) SECOND STAGE GRAFTING OPERATION. Donor: 7B5/3 female, L H S OPERATION. Mouse very light and there may have been some graft displacement. (Graft definitely trimmed).

19 MAY 1954 (42 + 12) Second stage graft uncontracted, and fully epithelialized, with clean separation of cuticle. Nevertheless appearance is very delicate and rapid deterioration is to be expected.

21 MAY 1954 (42 + 14) Still high degree of survival; some contracture.

24 MAY 1954 (42 + 18)

Breakdown about complete. Score as 17 days survival.

INJECTION OF FOETAL MICE (A to CBA) WITH ADULT SPLEEN
AND KIDNEY CELLS

EMB-157

13 JAN 1954 DONOR: Exactly as EMB-156.

RECIPIENT: Exactly as EMB-156.

See clearly & inject 6 17/18 day fetuses. 0.01 ml; injections all O.K. No mobilization. Spirit & germolene on skin.

15 JAN 1954 Healthy litter of 7 born - + 1 dead and mauled - overnight. Age at time of injection just under 18 days. (Birth ~~12/2~~ 1 1/2 days after injection).

3 MAR 1954 Test-operation.

Donor: Stock 9 A-line male. Standard grafts, but rather on the thick side and probably active. Thickness of grafts accentuated by the delicacy of the recipients' skin.

RHS operate all 7 mice in this litter.

15 MAR 1954(12) 6/7 total breakdown -- SEE PT-42 for further use.

1/7 shows 100% survival and firm white surface though adh.cuticle.

18 MAR 1954 /15/ This graft is still surviving, but it is being subjected to a mild reaction - some contracture, very slight scab, epithelium far from robust and no hair-growth.

25 MAR 1954 (22) Graft now fading out - progressive contracture and scabbing is taking place. There has been no hair-growth.

26 MAR 1954 (23) Breakdown may be taken as complete thisday.

IMPORTANT: See PT-42 (ineffectiveness of nodes).

25 NOV 1953 Donor & injection material: As EMB-141 q.v. (important).

Recipient:- CBA Stock V F X CBA Stock II male, 2nd preg., S.D., special EMB mating pots.

Inject 7 foetuses, 17/18 days (PBM thinks 17), each 0.01, with no doubts about working of syringe (cf. EMB-141). Visualization & injections excellent. Operation complete by 11.30 a.m.

26 NOV 1953 Litter of 6 (+ 1 dead) found this a.m. and presumably born overnight 25/26 Nov., i.e. 12-18 hr after injection.

8 JAN 1954 Test-operation.

Donor: B-line Stock 2 female. Operate RHS on all 5 survivors.

18 JAN 1954 (10) - Inspection. 1/5 Total breakdown. 1/5 very high degree of survival, but central weakness with peeling epithelium. 1/5 100% survival but rather pink; 2/5 100% survival and look very good. ? 2-3 supermice.

21 JAN 1954 (13 days)

Note the new system of numbering: 1. (R) F; 2. (both) M; 3. (2R) M; 4. (R) M.

1. (R) Graft fully surviving and uncontracted; but it is rather pink and has until now been rather swollen. There are one or two tiny scabs.
2. (both) Graft predominantly surviving and uncontracted, but pink and swollen, with small anterior and posterior scabs.
3. (2R) Graft largely surviving, but several largish scabs in centre and anteriorly. Some contracture.
4. (R) M Much contracture, and scabbing has spread over whole of dorsal 2/3. Ventral 1/3 surviving.

23 JAN 1954

- (15) 1, 2, and 3 still show high degree of survival.
4 has b.d. just about complete. KILL.

25 JAN 1954

- (17) 1. Graft is surviving, but scurfy, slightly contracted, and no sign of hair-growth.
2. B.d. just about complete. KILL.
3. B.d. far advanced.

29 JAN 1954

- (19) 3. B.d. completed. Kill.
1. Graft still hanging on, but no hair-growth and some scabbing posteriorly.

5 FEB 1954

- (26) 1. Graft is still hanging on, and is now growing a small tuft of black hairs. This is most encouraging and may be a sign that this graft is now settling down.

10 FEB 1954 (31) Graft is doing fine and is continuing to grow patches of black hairs which are now reaching a respectable length.

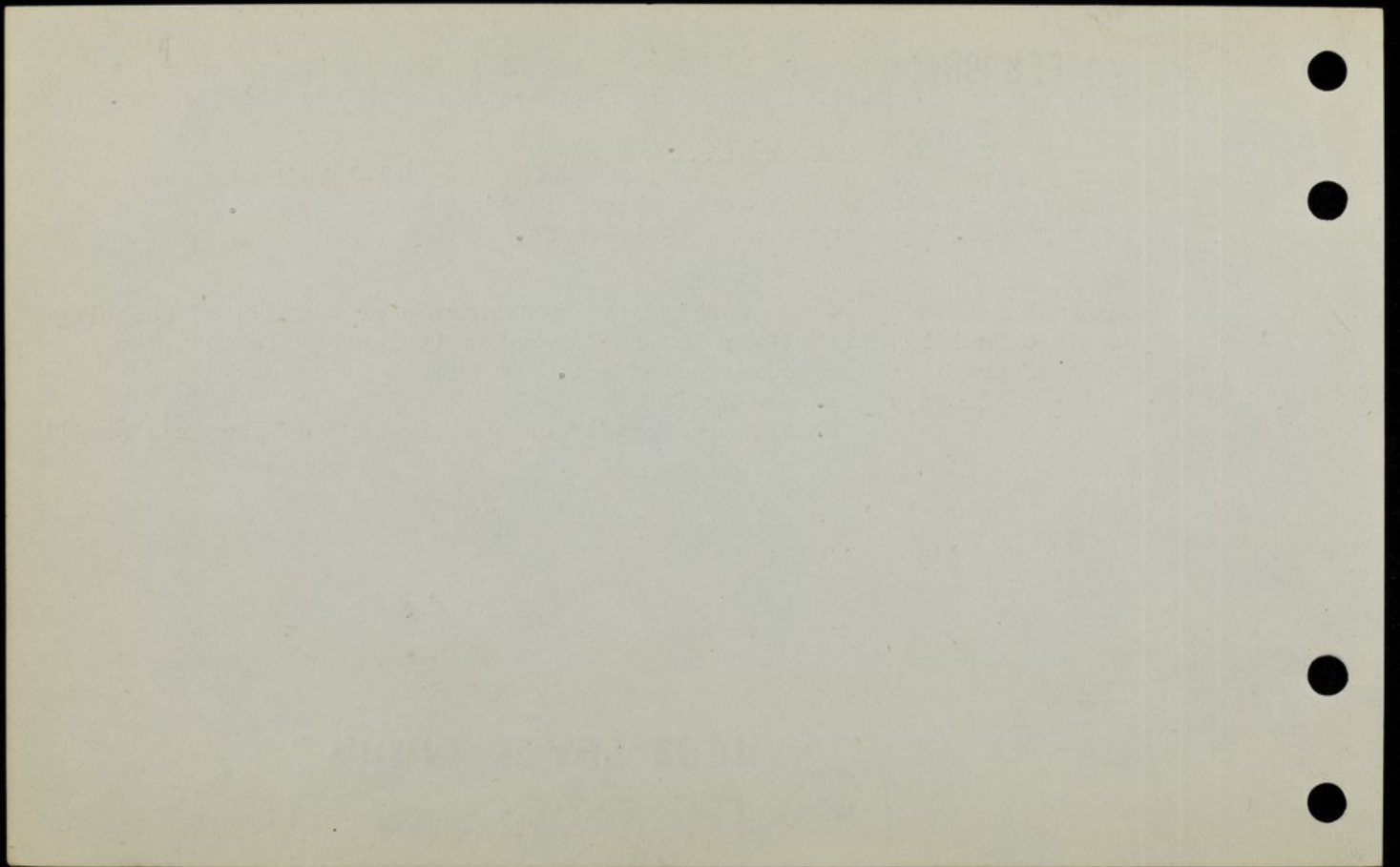
17 FEB 1954 (38) Reoperation.

Graft appears to be in place and is growing normal-length dark hairs (non-agouti) with postero-ventral orientation (about 45 deg.)

Regrart LHS. Donor: Stock 5 B-line male.

23 FEB 1954 (36 + 6) Healing of graft very poor - areas of scabbing but some healthy epithelium; the latter makes it improbable that the state of graft is due to immune effect.

Biopsy: a. new graft
b. old RTW "graft" or what is left of the area (small)



INOCULATION OF FOETUSES (B to cba) THROUGH BODY WALL

EMB-136.

14 OCT 1953

Spleen Kelly + Foster
Donor tissue: Exactly the same as for 132.

Recipients: Foetuses of cba Stock 2 female, special diet, multi-parous.

3 + 3 seen and injected. Age 16-17 days. Visua-
sation excellent, and injections reliable. 0.01 cc.

Very hopeful.

18 OCT 1953

Healthy litter of 6 born - age at time of injection there-
fore 16 days - most favourable.

22 NOV 1953

Several mice of this litter are somewhat runtish, but only
slightly so. It has been noted for some time now that 2 of the young mice
have abnormal hair colouration: they are decidedly more grey (lacking
much of the yellowness of normal cba's). Watch.

23 NOV 1953

Mark the 2 dark grey mice with R ear notches. Examine their
hairs using a lens, and compare with those of mother: they are very
dark, almost black, and the yellow pigment is almost completely absent
except at the very tips. The skin itself is very dark leaden blue and
has the appearance of intensely active skin, and this state of
affairs seems to prevail over the whole of the body-surface, including the
abdomen. The density of hairs too is abnormally high.
2 other mice are slightly darker than their normal equivalents.

9 DEC 1953 This litter is now approx. 7 weeks old. Note that the colour 'abnormalities' noted previously have disappeared - the dark hairs must have been due to intensely active skin.

Test operation.

Donor: Stock 2 B-line female - note that the original donor came from the same stock.

Recipients: 6 mice, standard grafts and standard RHS operations.

19 DEC 1953 (19) FIRST INSPECTION. All grafts well healed: one graft TBLs; a second graft (M, R notch): considerable haemorrhage, but partial survival. (This is one of the mice showing the apparently abnormal pigmentation referred to above.)

SUPERMICE:-

Male, L notch = 136.1 Graft perfect except for 2-3 tiny points of epithelial loss.

Female, - notch = 136.2 Graft perfect

Male, both ears = 136.3 Graft perfect: dorsal pigmentation.

Male, 2R notch = 136.4 Graft fully surviving, but perceptibly inferior to 2,3.

No plasters. Germolene around but not on grafts.

23 DEC 1953 (14) In general, very disappointing. Within a few hrs of deplastering on 19 DEC the grafts showed some deterioration, and in all cases there has been extensive contracture and other pathological changes over the past three days. At present, however, all show some survival and one has started to grow hairs.

- 1 (M, L notch). Localized superficial scabs, but graft centre, though bald and crinkly, mainly surviving.
- 2 (F, no notch). Graft reduced, bald and shiny, but ep. still firm.
- 3 (M, both ears). Much contracture, and most of ep shiny & bald; but three patches are deeply pigmented, and from these black hairs are just beginning to grow.
- 4 (M, 2R notch). The worst graft: reduced, puckered, scabbed and recessed. Still some slight survival.

24 DEC 1953 (15) There is no longer any ground for supposing that 4 has any surviving epithelium.

26 DEC 1953 (17) Graft on 136.1 now completely ulcerated & scabbed. Hair growth from other two grafts (black hairs).

(-20) 1. No survival. Survival score 17 days.

4. See note of 24th Dec. But it is just possible that

there is still some survival in a ventral horse-shoe shaped region.

2. Most of graft is hairless and with somewhat weak-looking epithelium, but anteriorly there are a few black hairs as well as some superficial scabbing which has already involved much of the hair-growth. Graft clearly still surviving, but only just hanging on.
3. Graft predominantly surviving, with 2 patches of black hair-growth dorsally. At the dorsal margin there is also a small scab, and the centre of the graft bears a small circular patch of redness. Graft surviving, but suffering from a chronic reaction from which it may not recover.

18 JAN 1954 (30)

2. Graft small but it appears to be in place. No hairs. Scurfy cuticle; tiny superficial serous scab in centre, and small fresh scab ventrally. Survival seems probable.
- 4 & 3. Graft very small, but appears to be in place, the epithelium looking quite healthy. It is difficult to know whether this area is graft remnant or healed scar tissue. - ~~it is rather too large for the latter to be very probable~~
3. & 4. ~~Small hairless area~~ Graft very small and appears to be in place, the epithelium looking fairly healthy. A few straggly and isolated hairs appear to be black, but it is ~~completely~~ not possible to be completely certain. Graft most probably surviving.

2 and 3 are most probably survivors.

All mice in this litter should be tested with a 2nd graft in due course.

29 JAN 1954 It would be foolish to score these mice as survivors after 30 days (8 Jan) since survival in 2 of the mice became a matter of conjecture after that date.

EMB-136

Approx scores for this litter are as follows:

17, 30 (?), 30 (?), 30 (?) days.

i.e. clear prolongation, but chronic and nagging reaction resulting in progressive contracture and fading out. Scores cannot be taken too seriously - ~~b&g~~ they may well be overestimated.

Regraft the whole litter when convenient.

17 FEB 1954 (70) Reoperation.

Donor: Stock 5, B-line male. Standard grafts. LHS operations. Inspect at 6 days. *Both ears: dead post-op*

23 FEB 1954 (70 + 6) Healing satisfactory except for dorsal half of one graft. No immune effect - high degree of survival. Rebandage.

27 FEB 1954 (10) L ear: B.d. complete or very nearly so - scabbing on exposure. - Kill.

R ear: Graft completely surviving - slight haemorrhagic pattern but otherwise O.K. - prognosis not too bad. Cuticle off cleanly.

No notch: As for R ear, no haemorrhagic pattern at all but graft very white. Small patch of scabbing after exposure indicates that b.d. is in fact in progress.

28 FEB 1954 (11) No notch has now broken down - scabbing over most of graft. Kill.

2R ear: Graft still surviving but severe contracture. This graft will fade out very gradual, but note the lack of severity of reaction.

-1 MAR 1954 (12) 2R ear: graft still present, but dontracture continuing. This graft is fading out, but as yet complete survival.

16 MAR 1954 /27/ Graft has persisted, though there has been antero-posterior contracture. About 485 days ago black hairs began to emerge from part of graft, and these hairs have now formed an area of bushy fur. The rest of graft is still bald, but it appears that hair-growth is slowly spreading. At any rate, the appearance of black hairs is clear evidence of the survival of graft epithelium.

5 APR 1954 (47) The hairs which have been growing vigorously in the last few weeks, have become less conspicuously black; examination of them with a lens reveals however that they are very dark compared with those of the host, and have no yellow tips.

Shave graft area: somewhat scary, but there can be little doubt that a narrow and rather elongated graft remains in place, with internal follicle pattern and dark pigmented patches. RHS:scar only. Reoperate in due course, but meanwhile assume that graft is viable,

EMB-136

14 APR 1954 (56) Graft has grown another crop of black hairs. There can be no doubt that the graft remnant is surviving. Another graft might be desirable.

This mouse has now been tolerant for
70 + 56 = 126 days.

(70 + 79) since first test graft.

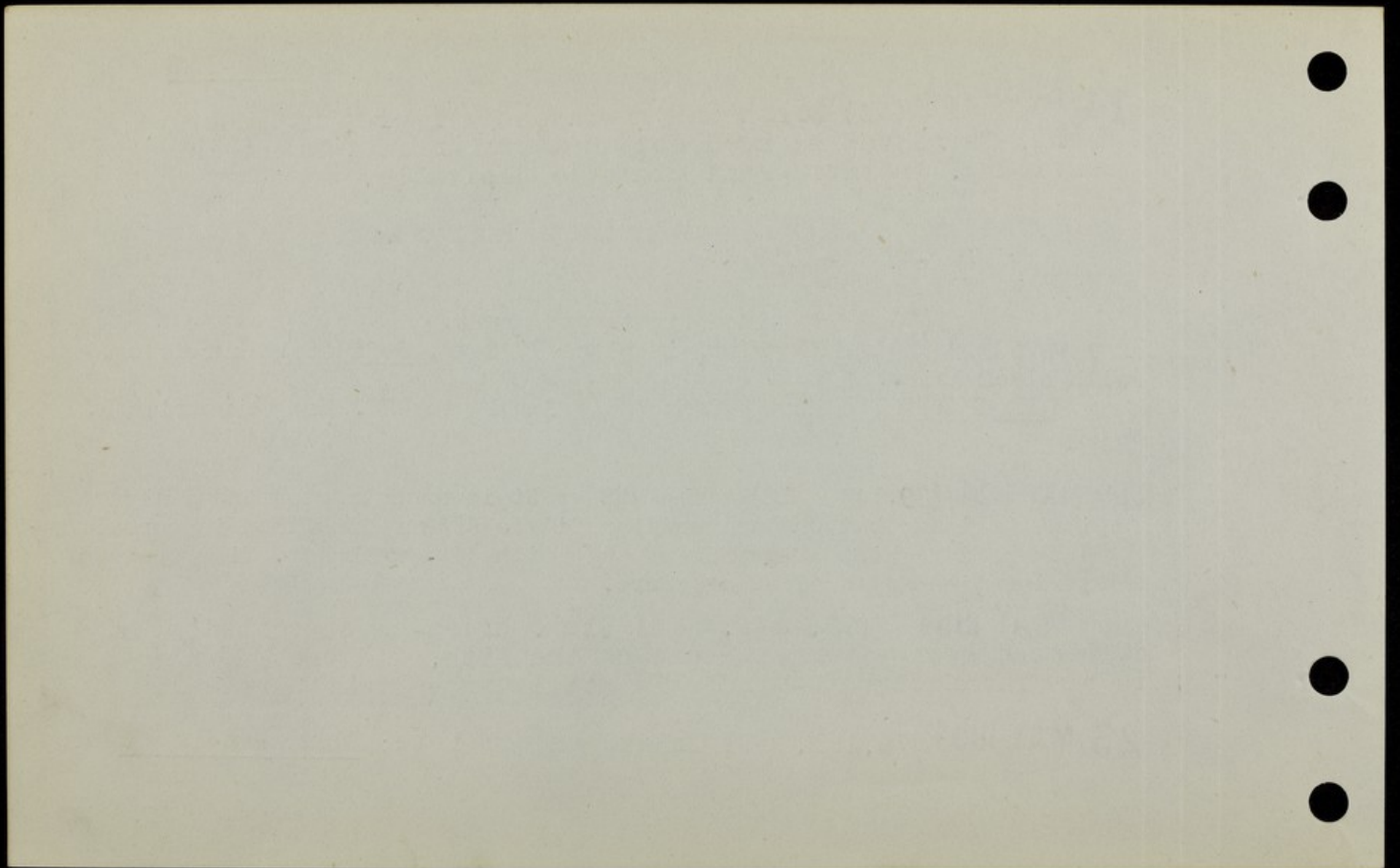
7 MAY 1954 (79) LHS graft, in place 79 days, definitely surviving, with black hairs.

RHS OPERATION: Graft from 7B5/3 female to RHS. May be untrimmed.

19 MAY 1954 (79, 149 + 12). The RHS graft is uncontracted and fully epithelialized; but the colour is a little darker pink than is normal, and there is a hint of puffiness and epithelial weakness. Tiny spots of deterioration appeared on exposure.

21 MAY 1954 (79, 149, 14) Slow, criss-cross scabbing commenced soon after exposure on the 19th, and breakdown is now far advanced - probably still very slight survival.

23 MAY 1954 (79, 151, 16) Breakdown complete: score as 15 days.



23 SEP 1953 Donor tissue: Same as for EMB-129.

Recipients: Foetuses of cba Stock 2 female, special diet, 1-parous, 40 g.

Visualisation very poor since the placentae are uppermost. Age of embryos about 16-17 days. 6 seen and injected; whilst the injections are not 100% reliable there is reasonable hope that most are intraembryonic. 0.01 cc.

25 SEP 1953 Litter of 7 born overnight - 5 dead and 2 alive. Depressing.

Note that the 3 survivors from EMB-131 have been added to this litter due to suckling failure of mother. These will probably not survive. They are not identifiable.

27 SEP 1953 3 survivors remain - 2 are bigger and more vigorous than the third and clearly these are the rightful young of this mother.

14 NOV 1953 Test operation.

The mice are now about 7 weeks old.

Donor: A-line Stock 7 male.

Recipients: 3 mice: 2 from EMB-130, and 1 from EMB-131. Standard RHS operations. Notch mice bear.

1953

24 NOV 1953 (11 = MST). One graft shows orthodox total breakdown.

The other two (notch L ear) show a high degree of survival. Of these two, the epithelium is definitely weak in one, for a tiny strip could be peeled away to expose a drying surface; at present the rest looks OK. In the other, the graft looks firm and solid and is thickly epithelialized; clean separation of ghost etc. **?Supermouse.**

26 NOV 1953 (12 = MST + 1). The graft on one of the two hopefuls (notch L ear) has broken down.

The other graft still survives, but it has contracted and has a hard outline. In the ventral third there is a dense but short crop of new white hairs; but the dorsal $\frac{2}{3}$ has an ominous yellowy tinge, though overt breakdown has not begun.

28 NOV 1953 The really hopeful mouse is turning out to be a full-scale **super-mouse** - it has established itself against slight initial resistance, and is now growing rather fine hairs over the greatest part of its surface.

29 NOV 1953 (16) Hair-growth over practically the whole surface now really convincing - a nice-looking graft.

22 DEC 1953 (39) PASSIVE TRANSFER EXPERIMENT. Excise & chop in usual way 8 (grossly hypertrophic) draining axillary nodes from four mice (CBA) immunized 11 days ago with A-line skin: see **11 DEC 1953** under **PT-30**. Load into standard pipette mounted trochar and introduce intraperitoneally. **Some bleeding after inoculation.**

The graft was clipped before implantation: it is immaculate in every respect.

24 DEC 1953 (39 + 2). No tangible change in the graft. Area $6\frac{1}{4}$ A/P x $6\frac{3}{4}$ D/V.

26 DEC 1953 (39 + 4) Graft 6 mm diam circle. The graft is now swollen and being perfectly symmetrical it is domed up above the level of the surrounding skin, and the swelling is soft & oedematous. There is a barely perceptible area of vascular dilatation medio-posteriorly, but at this stage none of the pathological changes would be held significant unless there was some prior reason to expect deterioration. The swelling is nevertheless perfectly clear-cut.

28 DEC 1953 (39 + 6) Graft $5\frac{1}{2}$ mm diameter circle. **Conspicuous pathological changes.** Graft is still more domed, but the swelling is not longer soft and freshly oedematous: the graft has hardened, is palpable, and tends to move as a whole. Scurfiness is pronounced, and there is a general angry reddening.

30 DEC 1953 (39 + 8) Further contracture: dimensions 4.5 mm A/P, 5.0 mm D/V. Graft very hard and palpable. The angry reddening and scurfiness is turning into antero-central mummification, and there is general yellowish discolouration.

31 DEC 1953 (39 + 9) Contracture continuing. Mummification has spread over most of graft, which is now grossly discoloured and very hard and scurfy. There is still some survival, but scabbing is very imminent.

Colour-photograph.

7 JAN 1954 (39 + 10) Dimensions now 4.5 mm A/P, 4.5 mm D/V. Small degree of survival - most of graft is mummified and about to scab.

2 FEB 1954 (39 + 11) The scabbing has spread over most of the graft and b.d. is just about complete.

B.d. complete 10 days after implantation of immune nodes.

17 FEB 1954 (39 + 11 + 46) Reoperation.

Regraft mouse on LHS. Donor: Stock 7 A-line male. Standard graft. Inspect at 6 days.

23 FEB : Healing good but surface very white - probably high degree of survival. Uncertain whether immune effect. Unfortunately mouse accidentally killed - hence take biopsy.

4 SEP 1953~~FOETAL~~ Donor tissue: Exactly as for EMB-124.Recipients: Foetuses of cba female from Stock 2. Special diet, mouse multiparous, weight 34 g.

6 embryos seen and injected - this includes one that was probably dead since it was paler in colour than the rest. Age about 15-16 days. Visualisation fairly good. Inject 0.05 cc.

8 SEP 1953

Litter of 4 born overnight, all looking very healthy. Age at time of injection therefore 15-16 days, as estimated.

27 OCT 1953Test-operation.Donor: A-line Stock 6 female, standard body-skin grafts.Recipients: All 4 mice of this litter, now weighing from 19-23 g. Operations standard RHS.**7 NOV 1953** (11 = MST). 3/4 show typical picture of very advanced or total breakdown. 1/4 shows 100% survival; surface a little delicate: supermouse?

It was intended to use the draining nodes from the 3 immunized mice for passive transfer exps, but on dissection, the draining nodes in 2

mice were abnormally small (in one of these the superficial node was not found at all); and in the third mouse the nodes were normal but not markedly hypertrophic.

- 9 NOV 1953 (13) Perhaps the graft on the one 100% survivor at 11 days has contracted a little, and though the surface is intact there is just a hint of redness in a dorsal patch. But the surface is intact, and hairs are just beginning to pierce the thicker (active) posterior margin. Unquestionably a prolongation of survival.
- 11 NOV 1953 (15) Graft still surviving: but still further contracture, and in spite of penetration of hairs there is a scaly reddened look about it. Obvious not a perfect super-graft, though prolongation conspicuous.
- 12 NOV 1953 (16) Graft still surviving, but it looks by no means healthy and contracture is probably continuing. Hair-growth is grossly retarded - there are a few hairs only near the antero-ventral margin.
- 13 NOV 1953 (17) There is now widespread central scabbing and the graft looks very much on the way out; but there is nevertheless clear-cut hairgrowth anteriorly, where the epithelium is still relatively healthy.
- 14 NOV 1953 (18) Some further deterioration; but hairs in place and still some surviving epithelium.
- 20 NOV 1953 (24) Most of the graft - practically all of it - has scabbed.
Neve

Nevertheless, a tiny islet of epithelium remains near the anterior margin, and this is actually bearing hairs & stumps.

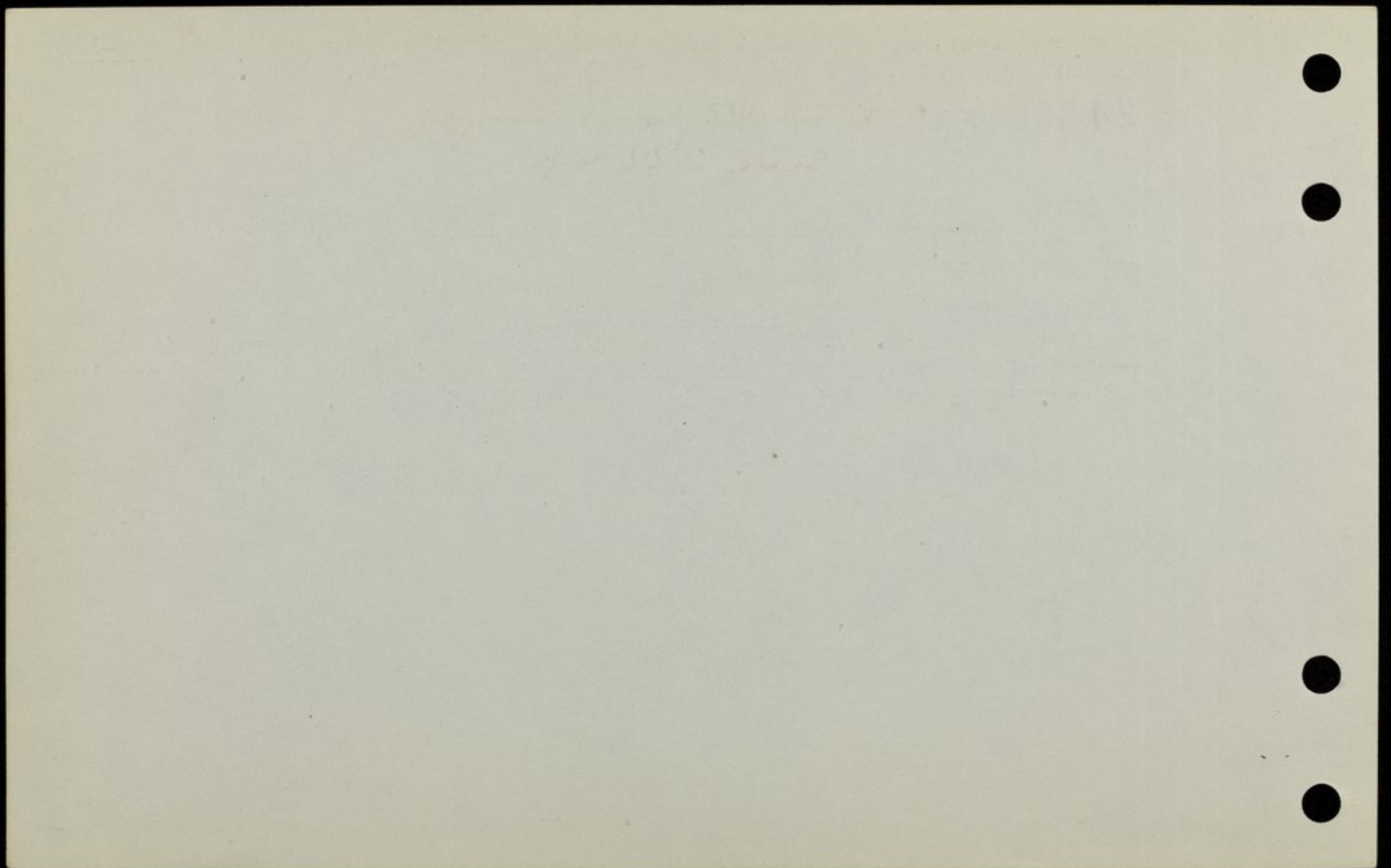
EMB-126

24 NOV 1953 (28) *Scabbing about complete.*
Score: 28 days.

3 DEC 1953 (28 + 9) Second stage graft. Donor: A VIII male. Normal skin.
LHS OPERATION.

14 DEC 1953 (37 + 11) First inspection of second-stage graft. The LHS graft well healed. At first, epithelium looked firm, but part of it peeled right away, & this part dried out in air in usual way. Nevertheless, approx. 50% of the epithelium is still firm & in place.

15 DEC 1953 (37 + 12). Breakdown nearly complete: S.T. can be taken as 12-13 days. Use in PT-32 exp.



INOCULATION OF FOETAL MICE (A to cba) WITH ADULT CELL
SUSPENSION THROUGH BODY-WALL.

EMB-115

4 JUL 1953 Donor tissue: Exactly the same as for EMB-114.

Recipients: Foetuses of female (cba) from STOCK 1. 33 g. No previous special diet. 0-parous.

3 + 3 foetuses seen and injected. Age about 16 days. Visualisation excellent, and injections satisfactorily intra-embryonic. A hopeful operation.

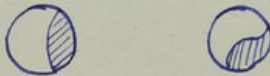
7 JUL 1953 Very healthy litter of 8 born overnight. This must be a 100% yield. Age at time of injection therefore 16 days.

26 AUG 1953 Test operation.

Donor tissue: Body-skin grafts from 2 Stock 111 A-line females.

Recipients: All 8 mice of this litter - i.e. a 100% yield for testing. Do not number. Weights are: 20, 20, 18, 17, 19, 18, 20, and 17 gs. Operations quite standard and satisfactory.

6 SEP 1953 (11 = MST). Six of these eight mice show typical MST picture in all respects, though in two the degree of survival is 25-50%, the survival pattern being shown by shaded areas on diagram:-



(PTO.)

The remaining two are impeccable in every respect: **SUPERMICE**. Leave unbandaged and undressed. One is male and the other female.

Use the 6 duds as under PT-11,12,13.

10 SEP 1953 (15)

1. (M) Graft in excellent condition; good crop of hairs developing.
2. (F) Animal pregnant. Graft has so far been more scurfy and a little more delicate than 1, but hair-growth is now beginning.

13 SEP 1953 (18) Both grafts are O.K. 2 has had healthy litter.

- 21 SEP 1953 (25)**
1. Graft in fine shape; hairs at 180 degs.
 2. Graft in fine shape; hairs have host orientation.

Reoperate both animals with A-line grafts (from Stock 5 female), this time on LHS. Standard opns. Smear old grafts with vaseline to prevent adhesion to plaster.

3 OCT 1953 (25+12)

1. RHS: graft continues to be in good condition and is little the worse for wear.
LHS: graft rather small, but soundly epithelialised and in good shape.
2. RHS: graft continues to be in good shape.
LHS: graft large and robustly epithelialised - very healthy.

7 OCT 1953 (25+14)

1. Both grafts are O.K. - the LHS is small but healthy, rather scurfy.

2. Whilst the RHS graft is O.K., the LHS does not look too healthy to-day. It is somewhat contracted, swollen, and has the shiny and bald look which is so characteristic of a mild reaction. There is also a small ventral - but very probably non-specific- scab.

Note that there is no recognisable sign of a reaction on the old graft: it may well be that the newly transplanted graft is able to pick up a very mild reactivity on the part of the host which does not show up in the old and well established graft. On the other hand, a non-specific cause (irritation due to the plaster followed by scratching etc) cannot be completely ruled out at present.

8 OCT 1953 (25+17)

1. RHS (old graft): perfectly O.K. - no trace of a reaction.
LHS (new): this graft is now very small; it has clearly gone through a very mild reaction and has undergone slow contracture; its surface is pink - quite healthy - but rather scurfy and there is only the beginning of hair-growth in the form of a few odd hairs. The behaviour of this graft is undoubtedly anomalous (cf. 2).
2. RHS (old): graft is O.K and without any visible signs of a reaction.
LHS (new): this is still slightly puffy, and has contracted to the size of a smallish graft (it is larger than that of 1 but it must be remembered that had been quite large originally); there is still no hair-growth but the epithelium is now looking more robust and it may be that the graft is beginning to settle down.

9 OCT 1953 (25+18) Both the new grafts seem to be recovering - both are now beginning to grow white hairs.

15 OCT 1953 (25+26) All 4 grafts are doing well and growing bushy pelts.

30 OCT 1953 (25 + 41) Litter of five from the female by the male. These mice were fully pigmented at birth, and were identically pigmented when inspected 10 days later. Just conceivably the pigmentation throughout is not as intense as normal.

..

9 NOV 1953 (25 + 51)

SKIN GRAFTING TEST

[Cf. also EMB-108 this day.] Under ether anaesthesia, take one graft from the L thigh of the (suckling) female, No.2, and the male, No.1. The graft from the female round, but **small and very delicate**. Graft from the male thick but not active. **Both recipients had active skin.**

Transplant each graft to a CBA Stock VIII male recipient:-

Graft from female, 115.2 to Notch R ear
- - male 115.1 - Notch L ear

20 NOV 1953 (11) Inspection of cba recipients carrying grafts from 1 and 2.

R ear: graft small, and healing not perfect but on the whole satisfactory.

Cuticle tenaciously adherent. Surface is epithelialised and O.K.

L ear: cuticle strongly adherent and cannot be removed. Graft roof looks O.K. but may be a little delicate.

21 NOV 1953 (12) R ear: graft is scabbing.
L ear: graft is predominantly viable, but ventrally there is a patch of necrosis; there are also a few points of weakness.

22 NOV 1953 (13) R ear: b.d. complete - it was probably so yesterday.
L ear: graft still viable, but there are dorsal as ventral patches of superficial scabbing.

23 NOV 1953 (14) L ear: the central part of the graft is so far holding its own and is not contracting appreciably; but it looks by no means happy.

26 NOV 1953 (17: Report on skin-grafting test of 9 Nov, continued).

Notch R ear recipient (graft from female, 115.2). The grafted area is represented by a scar only. Breakdown must have occurred at about the 12th day (see above). **Destroy this mouse.**

Notch L ear recipient (graft from male, 115.1). The graft is rather hard, perceptibly contracted, and with an incisive outline. There is a strip of active skin growing agouti hairs (pointing antero-ventrally) in a small postero-ventral strip inside the graft margin, corresponding to the scabbed area noted at 12 days. More dorsally, there is thin straggly agouti hair growth pointing more or less down. **Thus this graft has overridden a severe reaction of some kind, but now appears to have just about pulled through.**

28 NOV 1953 Notch L ear recipient: this graft is clearly in place and appears to be settling down, but hair-growth is still confined to a narrow and now almost circular strip which is particularly active and which corresponds roughly to these parts of the graft which were most severely affected by the earlier reaction.

(25 + 70) All 4 grafts on the EMB-115 mice, male and female, are doing well. The female had another litter born on the 23 Nov. All are normally pigmented. (4)

30 NOV 1953(98)

S P E C I F I C I T Y T E S T

INTRA-STRAIN GRAFTING TEST (REPETITION)

(A) Cut standard skin grafts from the normal* inactive skin of the RHS thigh of 115.1 & 2. (Graft from 115.2 (F) very thin).

-Transplant graft from ~~XXXXX~~ 115.1 (M) to 6C4/P male
- - - - - 115.2 (F) - 6C4/P female

Graft beds were of normal active skin. Somewhat open fit in graft from 115.2 upon 6C4/P F.

NOTE:- The mother of EMB-115 came from CBA Stock I. Supposing it not to be a member of the litter ex 2C3/6, it will be closely related to the 6C4/P recipients.

(B) Cut 2 $\frac{1}{2}$ -sized round graft from Stock IV B-line male; trim. These grafts had small patches of active hair growth.

On the recipients, close-clip the dorsal midline skin between the resident A-line grafts; shave after thickly vaselining the grafts. The A-line grafts on 115.1 male showed the faintest trace of redness & scurfiness, but so did the host skin between (semi-active). Grafts on 115.2 perfect.

The small B-line graft was in each case planted carefully between the two resident A-line grafts (therefore draining into same nodes, NB.), leaving good rim of normal host skin between the three.

3 DEC 1953

Final report on the recipient of skin from 115 male.

This graft is now 24 days old and is settling down to a perfectly normal existence on its cba host; hair-growth is now abundant and over the whole of its surface. Note that this graft, though now normal, had earlier gone through a mild reaction. KILL THIS RECIPIENT.

10 DEC 1953 SPECIFICITY TEST: FIRST INSPECTION (107,9,82).

1. (M) B-line graft: Breakdown in progress, with some scabbing, but still some survival.

Both A-line grafts are in good condition (though naturally scruffy due to the irritation of the plaster) and the

there is no evidence of a reaction.

2. B-line graft: ~~241122&22222~~ Breakdown in progress, and scabbing is taking place over most of its surface; there is however a small area with surviving epithelium.

Both A-line grafts are in pretty good condition, and there is no clear-cut evidence of a specific reaction. The grafts are somewhat reddened, but this is probably due to irritation of the plaster.

Since b.d. of the B-line grafts is not quite complete, take no biopsies to-day. COLOUR*PHOTOGRAPH BOTH MICE.

11 DEC 1953 (108,10,83) Specificity Test, cont.

115.1 (male). Central B-line graft reduced to scab, and subsequent removal shows total breakdown of very long standing. Resident grafts:- LHS (83) a tiny bit scurfy; RHS (108) has a reddish streak down the midline; it is a little scurfy and has at least one tiny plaque of dried serous exudate. At present, these changes could be non-specific.

BIOPSY:- Take central pinch biopsy of LHS graft, and excise the smaller central B-line graft. Fresh bleeding from donor area of former. Label: 115.1 SPEC.

115.2 (female). Central B-line graft at first looked as if it had rim survival, but in fact rim was mummified: T.B.L.S. Only abnormality in A-line resident grafts is some reddening; small dried serous plaque on RHS graft anteriorly, but also on host skin in neighbourhood.

BIOPSY:- Take central pinch biopsy from LHS graft, leaving hairs in place. Excise central B-line graft.
Label:- **115.2 SPEC.**

NOTE: Little or no evidence to show that at this stage there is any specific reaction in the resident ~~mutag~~A-line grafts. Results tenerally comparable with those from 106 q.v.

[11 DEC 1953] First inspection of second test of intra-strain grafts from 115.1 & 115.2 (11 days: see 30 NOV 1953).

6C4/P M recipient (from 115.1): Graft perfect

6C4/P F recipient (from 115.2): Graft very thin and translucent (mother still suckling), but fully & firmly epithelialized.

NOTE:-(See also 108.2). These results very strongly suggest that the homograft reactions seen in the first intra-strain grafting tests on both 115 & 108 were due to intra-strain incompatibility as a result of using divergent sublines.

[14 DEC 1953] Specificity test: follow up (111, (13), 86). The resident

RHS (111) and LHS (86) A-line grafts are fully epithelialized and are recovering from effects of plastering etc. Either the minor pathological changes seen at 9 days were non-specific, as is very likely; or any specific change has now subsided.

Second inspection of intra-strain grafts from 115.1 & 115.2 (14 days after grafting).

6C4/P M (from 115.1 M): Grafts just possibly reduced; surface shiny & flat with cuticular exfoliations in form of little platelets. Hairs about to penetrate: have done so in one tiny area.

6C4/P F (from 115.2 F): Median ventral scab; rest of graft reduced & very shiny. Evidently some reaction.

17 DEC 1953 (114; 16; 89) Specificity test: all 4 grafts are doing well - the 2 LHS grafts have almost completely healed biopsy sites, and they have contracted correspondingly.

18 DEC 1953 (115, 17, 90). Specificity test:- 115.1 (M) RHS almost completely bald; fully & firmly epithelialized; patch of deep seated reddening in centre. LHS: patches of hairs ventrally; rest bald but firmly epithelialized & looks pretty healthy. Possibly v. slight reddening.

115.2 (F) RHS:- Long wispy hairs but bald in patches; fully & firmly epithelialized, but slightly pink. LHS:- Few hairs, otherwise as for RHS.

23 DEC 1953 (120, 95) Inspection.

- 115.1 (M) Both grafts perfect, with extensive new hair growth.
RHS graft $7\frac{1}{2}$ AP, $9\frac{1}{2}$ DV.
- 115.2 (F) Both grafts in very good condition, though RHS is just slightly and uniformly pink, with only small areas of new hair growth & slight shininess anteriorly. RHS size:-
 $7\frac{1}{2}$ AP, $9\frac{1}{2}$ DV. LHS also shows patchy hair growth.

In both mice, no graft has undergone contracture except that brought about by the now perfect healing of the LHS biopsy donor sites.

IMPLANTATION OF NORMAL CBA NODES

DONORS:- 4 mice from the first litter ex EMB-115, b.30 OCT. Excise & clean axillary, inguinal & cervical nodes from both sides of each. Divide into 2 sets of 19 nodes each, and chop for implantation in the usual way. Inject one set i/p into 115.1 and the other set into 115.2. Clean insertions.

(Node dosage approximately that used for PT experiments in supermice, after making allowance for the small size of the present nodes.)

Postoperative note: The female was repeatedly wetted over the abdomen during a difficult recovery period, and it seems certain that the bladder has been perforated. If the animal survives, it should be borne in mind (a) that the implants may have entered the bladder, which is not at all likely; or (b) that the explants will

be seriously damaged wither by urine in the peritoneal cavity or by the peritonitis that may come in train. Hence a negative result, if the mouse survives, may not be significant.

23 DEC 1953 (23) Final inspection of second test on intra-strain grafts from 115.1 & 2.

6C4/P M (from 115.1 M): Vigorous hair growth; graft normal-looking but highly active.

6C4/P F (from 115.2 F): Graft much reduced in area with general shiny and scar-like appearance; a central depression marks old scab. Hair growth only on perimeter, and almost certainly not of graft origin. A most severe reaction.

SUMMARY OF INTRA-STRAIN GRAFTS FROM SUPERMICE

<u>From</u>	<u>To</u>	<u>1st test</u>	<u>2nd test</u>
115.1	CBA VIII	Mod.reaction	Mod. reaction
115.2	do.	Breakdown	Severe reaction
108.2	do.	Mod.reaction	Minor reaction

In the 2nd test, the hosts were chosen for the closest known affinity to the supermice. The 'mod' reactions are no worse than intra-strain grafts from normal mice across wide gaps; only the behaviour of grafts from 115.2 is anomalous. The results just justify very accurate repetitions.

of these exps., looking for tendency for tolerated grafts to acquire antigenic props of host and of host tissue to acquire antigenic props of grafts. In effect this means that both the tolerated grafts & the general body skin of supermice should be transplanted both to their own strains and to the opposite strains.

26 DEC 1953 (+ 3) Implantation of normal nodes. Both mice are O.K., and all four grafts show further hair growth since the clipping 3 days ago. Check this & follow it up, as it provides an excellent qualitative distinction between implantation of normal & immune nodes.

28 DEC 1953 (+ 5) Ditto. Further vigorous hair growth. These grafts must be clipped fairly soon to see deterioration in due course.

30 DEC 1953 (+ 7) Ditto. 1. (M) This graft was close-clipped yesterday, and even since then there has been further noticeable hair-growth. Graft perfectly normal; no change in dimensions.

2. (F) Graft has not been clipped since implantation of nodes, and has a dense bushy white fur. A small scab is almost certainly non-specific on the RHS; the LHS too is in excellent shape. Do not clip until graft has been photographed.

31 DEC 1953 (+ 8) Colour-photograph both mice. Then clip 2: graft O.K. but there is a small scab which however spreads to the host skin (mites). Note that the LHS graft is perfect - hence no reaction.

11 JAN 1954 (+ 9) All grafts are O.K. - no sign of a specific reaction.
No change in dimensions. The small scab on the LHS of 2 seems to be
healing - powder with gammexane.

REPORT ON SPECIFICITY HISTOLOGY.

Both the B-line grafts showed abject breakdown, without the slightest
epithelial survival. B.d. somewhat abnormal - a marked black band effect
between the graft and its bed. This may be due to the fact that the grafts
were removed 1 day after the MST, and ~~after~~ after exposure to air had produced
hard scabs.

Both the A-line biopsies were completely normal, and without any signs of a
specific reaction,

THIS EXPERIMENT SHOWS CLEARLY THAT, WHEN TOLERANCE IS COMPLETE, THE PROCESS
IS STRICTLY SPECIFIC.

12 JAN 1954 (+ 12) [IMPLANTATION OF NORMAL NODES, cont.]

115.1 (M). Both grafts show localized superficial scabs, light brown in
colour; RHS graft has orangy discoloration and is soft
swollen, though not mobile 'as a whole'. The discoloration
is not even but rather streaky. Size: 7.0 A/P x 8.0 D/V.
This graft showed no pathological change on 2 JAN 1953.

115 2 (F). Early pregnant. A thin harvest of hair had to be clipped away,
representing growth since last clipping. The RHS graft has
a just perceptible orangy discoloration and slight very soft

This probably represents the first day on which any pathological change could be detected.

6 JAN 1954 (+ 14) (Implantation of normal nodes cont.)

1. (M) Further deterioration - scabbing and discoloration is more pronounced in both grafts. But as yet high degree of survival. Size: ~~8.0 mm A/P, 8.0 mm D/V~~ 6.25 mm A/P, 8.0 mm D/V Slight further contracture.
2. No change since last inspection. Graft is really in pretty good shape - one or two tiny superficial serous scabs which may or may not be non-specific. Size: 7.5 mm A/P, 8.25 mm D/V Very slight further contracture.

8 JAN 1954 (+ 16)

1. Further contracture: 5.0 mm A/P, 7.5 mm D/V
General deterioration continues, but still good and unquestionable survival - at least 50%.
2. Graft is O.K. - a little shiny in patches, but no clear-cut specific changes. Size unchanged (almost): 7.5 mm A/P, 8.5 mm D/V

9 JAN 1954 (+ 17)

1. Degree of survival still fair.

10 JAN 1954 (+ 18)

1. To outward appearance, still a small amount of sound epithelium on the RHS graft; but in fact the whole graft surface seemed mummified, with full-thickness scabs. Nevertheless, for consistency with other naked eye readings, the survival time should be taken to be 19 days. Thus breakdown span is 11 to 19 days.

2. RHS graft $7\frac{1}{2}$ + A/P X 8 D/V. This mouse gave birth to 4+ young yesterday. There has been no further hair growth, but pregnancy could account for this. The grafts are soundly epithelialized, but look a bit thin & translucent (pregnancy again). There is certainly a slight degree of swelling & an orangy tinge about the RHS graft, which might indicate the beginnings of a specific reaction.

13 JAN 1954 (120 + 21)

POST-IMMUNIZATION GRAFT ON X0 115.1. Donor:- A-line VIII male.
Low LHS operation.

Note: The two grafts on 115.2 show no further hair-growth (suckling); although tiny superficial scabs are present, and the grafts do not look very solid, there is no definite evidence of specific degeneration.
RHS graft: $7\frac{1}{2}$ A/P by 8 D/V, i.e. no change.

16 JAN 1954 Test-operate ex-115 litter of 5. Standard opns., RHS.
Donor: Stock 8 A-line male. Use this litter for Pt-38 at
MST

19 JAN 1954 6-day inspection of 115.4. : second stage graft shows typical and violent immune effect - maceration and scabbing on exposure - no survival.

This result is in complete agreement with that of 108.2 and indicates that the breakdown of the first graft (super) was due to cells of the implanted nodes which, in some form - probably in the host's own nodes - continue to survive and which have now produced a normal secondary response.

21 JAN 1954 Report on grafts on 115.2.:
Size of RHS graft: $7\frac{1}{2}$ A/P, $7\frac{1}{2}$ D/V - i.e. there has been little further contracture since the last inspection. Yet there has been contracture, though only in a D/V direction, since the first implantation of nodes. The graft has a small ventral scab, and its anterior region in particular looks shiny and delicate.

There could be a mild nagging reaction resulting from the survival of a small amount of the inoculum; but at no stage has there been evidence of a clear-cut reaction with progressive scabbing.

27 JAN 1954(11) MST report on test-operation of litter ex-EMB 115.
Typical MST picture: no enhanced tolerance. See PT-37 & PT-38.

2 FEB 1954(161,136) PASSIVE IMMUNIZATION OF 115.2 WITH LYMPH NODE CELL SUSPENSION.

Note on grafts:- The grafts clearly show 100% survival, and both are much of a size: RHS $6\frac{1}{2}$ AP x 7 DV; LHS - $7\frac{1}{2}$ DV x 6 AP. There has been no further hair growth however, and a slightly pinkish colour persists. There are also the tiny blemishes that have been present since even before the implantation of normal nodes (attributed to mite irritation).

Inoculum: Take 10 nodes from the five immune mice of EMB-148 q.v. at MST + 13 = 13 days. These are 'failed supermice', and the grafts have completely broken down, but it should be borne in mind that they may not be fully immune. The nodes were small and not very milky on removal.

Using normal citrate-saline throughout, cut up into small pieces and by gentle but firm expression with a flat pestle (no grinding) combined with sucking in and out, prepare a node milk suspension which was then passed through a No.1 glass filter. Recover 2 ml suspension, which certainly consists of 80% of the node juice plus all the fluid in which they were extracted. In view of uncertainty about efficacy of immunization a negative result should be disregarded.

Inject the 2ml node suspension intraperitoneally.

4 FEB 1954 There has been slight deterioration of the small scabs on the RHS graft, but the change is not sufficiently pronounced for it to be ascribable to the injected nodes. Slight contracture:
RHS 7.0 A/P x 7.0 D/V; LHS 6.5 A/P x 6.0 D/V

6 FEB 1954 (136,161 + 4). (115.2)

RHS: Superficial scabbing spreading over anterior $\frac{1}{3}$ rd of graft. Real deterioration since implanting node suspension. $5\frac{1}{2}$ A/P X $6\frac{1}{4}$ D/V.

LHS: Slight reddening, scurfy surface. 6 A/P x 8 D/V.

8 FEB 1954 (136,161 + 6)

RHS: Scabbing has is very much more extensive than at 4 days and has engulfed more than half of the graft, which is now in very bad shape.

LHS: Still fully epithelilaised and supple. The reddening has deepened somewhat, and there are 2 tiny scabs - otherwise graft is in surprisingly good shape.

10 FEB 1954 (136,161, +8)

RHS: Scabbing almost complete - a tiny patch posteriorly may contain surviving cells.

LHS: There is now an extensive central patch of scabbing which has appeared very ~~clearly~~ quickly - but as yet marginal survival is fair. This graft is undoubtedly breaking down more slowly than the RHS but there may not be much in it in the end.

11 FEB 1954

(.....+ 9)

RHS: TBLS - peel off dried scab.

LHS: Breakdown just complete. The scabbing has spread with surprising rapidity, and it is just conceivable that there is trace survival along the posterior margin. But unless this is confirmed to-morrow breakdown should be scored as from to-day.

Note the tempo of the reaction compared with that produced by implanted immune node fragments. This squares perfectly with the node suspension result in the PT series.

12 FEB 1954

(..... + 9)

LHS: there is clearly TBLS. R.E.B. concurs.

i.e. breakdown of graft 2 days after implantation of immune node suspension; fastest yet.

17 FEB 1954

2nd stage graft.

Donor: Stock 7 A-line male. Standard graft. LHS operation.
Inspect at 6 days.

23 FEB 1954

(6) Strong immune effect - little survival if any. Kill.

INJECTION OF FETAL MICE (aaUU to CBA) WITH ADULT CELL
SUSPENSION THROUGH BODY WALL.

EMB-103

13 MAY 1953 DONOR TISSUE: The now standard dense adult cell suspension (on this occasion very dense: no settling at all) from testis, kidney, spleen and liver fragment of male mouse of litter 5B3/1 (b.21 Feb). **

RECIPIENT: CBA mouse, 4C4/2, b.2 Feb., wt. 34 g., pregnant (second pregnancy) by litter mate; special diet since May 1, segregated yesterday. Both ears notched.

3 +1 fetuses very near term; each 0.01 ml. Injections of all intraembryonic; of one, probably intrathoracic. Skin prep. by dry shave, no soap, + germolene.

** Two smears from brei.

Dry-weight determination: measure out 15 turns (0.15 ml) by micrometer syringe upon carrier of lg. torsion balance. Desiccate at 37 deg.C and reweigh.

Wt of carrier A	102 mg
A + 0.15 ml brei = B	248 mg
C = wt. of brei, B - A	146 mg
D = wt. after 24 hr over	
P ₂ O ₅ at 37 deg C.	112 mg
E = ditto, 48 hr	112 mg
Dry weight of 0.15 ml brei..	10 mg
Water in ditto, B - E	136 mg

*60deg C.

Summary: 0.01 ml injection suspension = 0.67 mg. dry weight

14 MAY 1953 (28 hr) Litter of 4 born 7 p.m. approx. These still healthy the following morning. (This represents a complete yield, including the embryo probably injected into thorax.)

1 JUN 1953 3 survivors remain. ✓

29 JUN 1953 Test operation.

Litter about 7 weeks old.

Donor: B-line male from STOCK 1 - standard grafts.

Recipients:

1. M (R) 20 g.
2. M (L) 20 g.
3. M (no) 18 g.

10 JUL 1953 (11)

1. Graft dry, white, epithelialised, supple, without any sign of a reaction except for inflammation of the anterior margin. Germolene. Possibility of short-term supermouse.
2. As for 1. but without the slightest sign of a reaction. Supermouse? Germolene.
3. Typical breakdown. Kill.

11 JUL 1953 (12)

1. Haemorrhagic scabby patch anteriorly. Rest with slight discolouration. But graft as whole surviving. Prognosis not too good.
2. Slight superficial scabbing dorsally, but rest of graft is O.K.

13 JUL 1953 (14)

1. Scabbing completed. Survival score ~~13-14~~ days. Kill.
2. Dorsal scab more pronounced - rest of graft shiny but perfectly O.K. and clearly surviving.

14 JUL 1953 (15) 2. Dorsal scab larger, small ventral scab, but large central region clearly surviving. No hairs. Surface shiny.

15 JUL 1953 (16) No change.

17 JUL 1953 (18) The dorsal scab is becoming free and has obviously reached the limit of its size. There is still a small ventral scab, but a fairly large central area is unquestionably surviving. No hairs. There is still some possibility that this graft may recover.

18 JUL 1953 Mouse 103. Further contraction, but still some central survival.

20 JUL 1953 (21) Scabbing is continuing, but there is still some slight central survival.

21 JUL 1953 (22) Scabbing complete. Survival time therefore 21 days.
Kill.

INTRAEMBRYONIC INOCULATION OF FOETAL MICE WITH ADULT
CELLS THROUGH BODY WALL (A to aaUU).

EMB-102

8 MAY 1953 DONOR: Injected material exactly as for EMB-100 q.v.

RECIPIENT: Credentials, parity & diet exactly as for EMB-100 q.v., but weight 29 g.

Visualization moderately good. 4 + 3 foetuses, about 16-days (and anyhow older than in EMB-100,1. At least four were injected successfully, and the other three are very likely to be intramebryonic also.

12 MAY 1953 Healthy litter of 6 born overnight. Age at time of injection therefore 16 days.

1 JUN 1953 All 6 alive, but 2 are very runtish and will probably not survive.

8 JUN 1953 One of the runts has died, leaving 5. This animal had clearly been well (too well!) injected.

30 JUN 1953 Test operation.

This litter is now about 7 weeks old. Operate only 4, leaving one runtish mouse to come.

Donor: Standard grafts from female of litter 4A4/3 (12 Feb.) and 5A3/3 (8 Mrach).

Recipients:

1. M (R) 22 g.
2. M (L) 24 g.
3. M (both) 25 g.
4. F (no) 17 g.

9 JUL 1953 (9 = MST). Nos.2,3:- Total breakdown or far advanced reactions. Destroy.

Nos.1,4 show 100% epithelial survival and are clearly supermice. However, No.1 has a rather delicate and slightly flushed appearance, and may be in throes of very feeble reaction. No.4 looks firm & solid, with matt surface.

Apply germolene to both.

Late afternoon: No.1 has several lines of deep red haemorrhagic scarring, but graft as whole clearly surviving. Mild reaction in progress.

10 JUL 1953 (10)

1. Considerable contracture has taken place, and the graft is scabbing rapidly and progressively. Yet a few patches of surviving epithelium remain. Breakdown will soon be complete.
4. Graft has contracted somewhat, slight marginal dorsal scab, delicate epithelium, thin flaky cuticle.

11 JUL 1953 (11)

1. Central thin scab lifting off. Good annulus of clearly surviving epithelium. Margin quite incisive - hence overgrowth unlikely.
4. Much as yesterday.

13 JUL 1953 (13)

1. Central scabbing continuing, narrow rim of epithelium surrounding it - rim with incisive external margin and could therefore be marginal graft tissue. But this seems highly doubtful.
4. Graft has contracted into a very small remnant. There could just be some survival.

14 JUL 1953

1. Scabbing complete. Kill. Survival score 13 days.
2. " " " " " 13 days.

16 JUL 1953 (69 days). Test operation on 102.5. Standard RHS graft of skin from late-pregnant A-line Stock II female. (Wt. 16g).

25 JUL 1953 (9)

5. Graft epithelialised and probably with 100% survival, but extensive dorsal haemorrhages do not augur well for the future. Rebandage. Epithelium very delicate.

27 JUL 1953 (11) Graft fully surviving, and epithelium seems to be more robust than at last inspection. Dorsal haemorrhagic patch still visible but gives impression of subsiding. Thin cuticle all over graft surface - peel off. Germolene. This graft, whilst it is going through a phase of specific reaction, may yet turn out to be a supermouse.

29 JUL 1953 (13) Graft looks pretty healthy now - the haemorrhage noted previously has very nearly disappeared and it is covered by robust and healthy epithelium; no significant contracture; scurfy cuticle; as yet no hairs.

31 JUL 1953 (15) Graft in good condition, but as yet no hair-growth.
Photograph.

6 AUG 1953 (21) Graft shows marked contracture, and has a small scab just dorsal of centre. Graft surface is white, smooth, hairless: no distinguishable dermal pattern. Yet area as whole probably too large to be a scar. Some prolongation of survival, but not very super.

8 AUG 1953 (23) Graft has now central scab with peeling epithelium; around this there is a pattern of radial puckering. Breakdown either complete or nearly so: for life table purposes score as 'greater than 20, less than 30 days'. **DESTROY.**

INTRAEMBRYONIC INOCULATION OF FOETAL MICE WITH ADULT
CELLS THROUGH BODY WALL (A to aaUU)

EMB-100

8 MAY 1953 DONOR: Albino male of litter 5A3/4 b.4 April. Remove spleen, 1 testis, 1 kidney (no liver) and prepare dense cell suspension by spin method.

RECIPIENT: 6 approx 15 day foetuses comprising the first pregnancy of B-line female 5B3/1 (b.21 Feb), no notch, pregnant by litter mate. Special diet from 6 April. Usual method (germolene etc). 30g.

Visualization poor, and embryos too young for best results; probably the injections were intraembryonic, but at least one was blind.

12 MAY 1953 Healthy litter of 6 born overnight. Age at time of injection therefore about 16 days.

1 JUN 1953 All 6 alive, but 2 are runtish.

8 JUN 1953 One of the runts has died, leaving 5. This animal had presumably been 'thoroughly' injected!

27 JUN 1953 Test operation.

Litter about 7 weeks old. Operate only 2 mice since the rest are very backward and underweight.

Donor tissue: Standard grafts from female of 4A2/3, b.12 Jan.

Recipients: 1. (R) M 25 g.
2. (L) F 18 g.

6 JUL 1953 (Ø)

1. Epidermis peels - moist surface, haemorrhagic blotching. Total breakdown. Kill.
2. Surface moist - haemorrhagic blotching. Total breakdown. Kill.

9 JUL 1953 Second test-operation.

Donor graft as for EMB-108.

Recipient is one of the remaining 3 runtish animals, weight now 18 g.

Operation RHS and quite standard.

The other two mice are coming on very well but are as yet too light to be operated.

18 JUL 1953 (9) 3. Graft had a delicate appearance; there is a patch of surviving epithelium anteriorly (hence no immune effect), but the posterior half is grossly ulcerated. Not a supermouse. Destroy.

28 JUL 1953 Third test-operation.

Donor grafts from A-line males from Stock 3.

Recipients are nos. 4 and 5 of this litter. Standard RHS opns,

4. Animal now weighs 19 g.
5. Mouse still an extreme runt and unlikely to make much further progress; weight 12.5 g. Operate in usual way, but use small plaster. Mouse very deep and unlikely to recover.

29 JUL 1953 The runt was found almost dead this morning; remove plaster bandage and allow to recover in incubator. The graft is in fact pretty firmly attached to the panniculus and its fate has probably not been prejudiced. Give animal a very small and loose-fitting plaster.

6 AUG 1953 (9 = MST).

100.4 Sound healing. Anteriorly, there is a patch of diffuse redness, and on exposure to air there was some slight puckering in this region; but the rest of the graft is perfectly sound, white and firm.

Prolongation of survival is certain, but a specific reaction may be in progress.

100.5 (Runt.) Healing OK though graft elongated in DV direction. There were little red spots on graft; epidermis partially peeled, to give surface drying in air to raw beef colour. About 50% survival only. Breakdown almost certainly imminent.

7 AUG 1953 (10)

100.4 Some contracture, and a number of tiny scabs; but predominantly a surviving graft.

100.5 Graft now wholly scabbed. Destroy.

8 AUG 1953 (11)

100.4 Further deterioration & contracture, but still 50% approx survival.

10 AUG 1953 (13).

100.4 Graft very white in colour but graft epithelium still survives. Dermal pattern of graft very prominent. There is a small lesion in the graft anteriorly.

24 AUG 1953 (27) Graft badly scabbed both dorsally and ventrally. There is possibly a small remnant of surviving epithelium in the central region. No hairs.

24 AUG

24 AUG

INOCULATION OF FOETAL MICE (A to cba) WITH ADULT CELL SUSPENSION
THROUGH BODY-WALL.

EMB-94

4 MAY 1953 Donor tissue: Exactly the same as for EMB-93.

Recipients: Foetuses of female from litter ex 2C3/6, b.2 Feb., pregnant by 4C1/2 or 4C2/2, probably o-parous, from stock-pot, special diet only since 2 May.

Foetuses very near term; 3 seen and injected intra-embryonically, one of them probably subcutaneously. Opn. satisfactory but foetuses probably too old. Inject 0.01 cc into each.

5 MAY 1953 Healthy litter of 4 born between 9 and 10 this morning. It is unlikely that there were more than 4 at time of operation. ~~When the foetuses~~

8 MAY 1953 3 lively youngsters remain. The 4th has vanished. ✓

27 JUN 1953 Test operation.

Liter about 7 weeks old.

Donor: Standard grafts from female of litter 4A2/3, b.12 jan.

Recipients:

1. M (R) 21 g.
2. M (L) 21 g.
3. M (no) 22 g.

8 JUL 1953 (11 - MST)

1. Healing perfect, graft soft, supple, pink, and well epithelialised. Clearly 100% survival. **Supermouse.** Germolene only.
2. Exactly the same as for 1. **Supermouse.**
3. Breakdown far advanced or complete. Kill.

Note that 2 is the maximum number of supermice to be expected from this experiment.

10 JUL 1953 (13)

1. Contracture is gradually taking place, epithelium rather delicate, graft pink, supple, with flaky cuticle. No hairs. Very small haemorrhagic patch. Mild reaction - prognosis poor.
2. As for 1. but without signs of haemorrhage.

11 JUL 1953 (14) Both grafts are much as yesterday.

13 JUL 1953 (16) 1. Graft much as before - only very slight contraction if any since last inspection. Delicately epithelialised, shiny, pink, no hairs, small flaky scab posteriorly, but dermal pattern is now visible. Graft seems to be holding its own at last.

2. Much as 1 but somewhat more contracted, epithelium more delicate, very small point of ulceration dorso-posteriorly.

14 JUL 1953 (17) 1. Some further contraction has taken place, graft now smaller than 2. Epithelium still shiny but possibly less delicate. Flaky cuticle, 2 small scabby patches, no hairs. Supple.

2. Now larger than 1, hence probably no further contraction since yesterday. Ulcer has healed (spot is delicately resurfaced), epithelium is generally shiny and on the delicate side. Yet the graft is unquestionably surviving and is by no means unhopefu. Supple.

15 JUL 1953 (18) Little change in either graft.

17 JUL 1953 (20) 1. Considerable contraction has taken place, and the graft is now well on the way out. Its surface is scabby-scurfy, but there is still a patch of clear-cut survival.

2. There has been further, if slight, contraction.

The epithelium has a scurfy cuticle and is clearly surviving. No hairs.

18 JUL 1953 (21) 1. Scabbing complete. Destroy. Survival : 20 days.

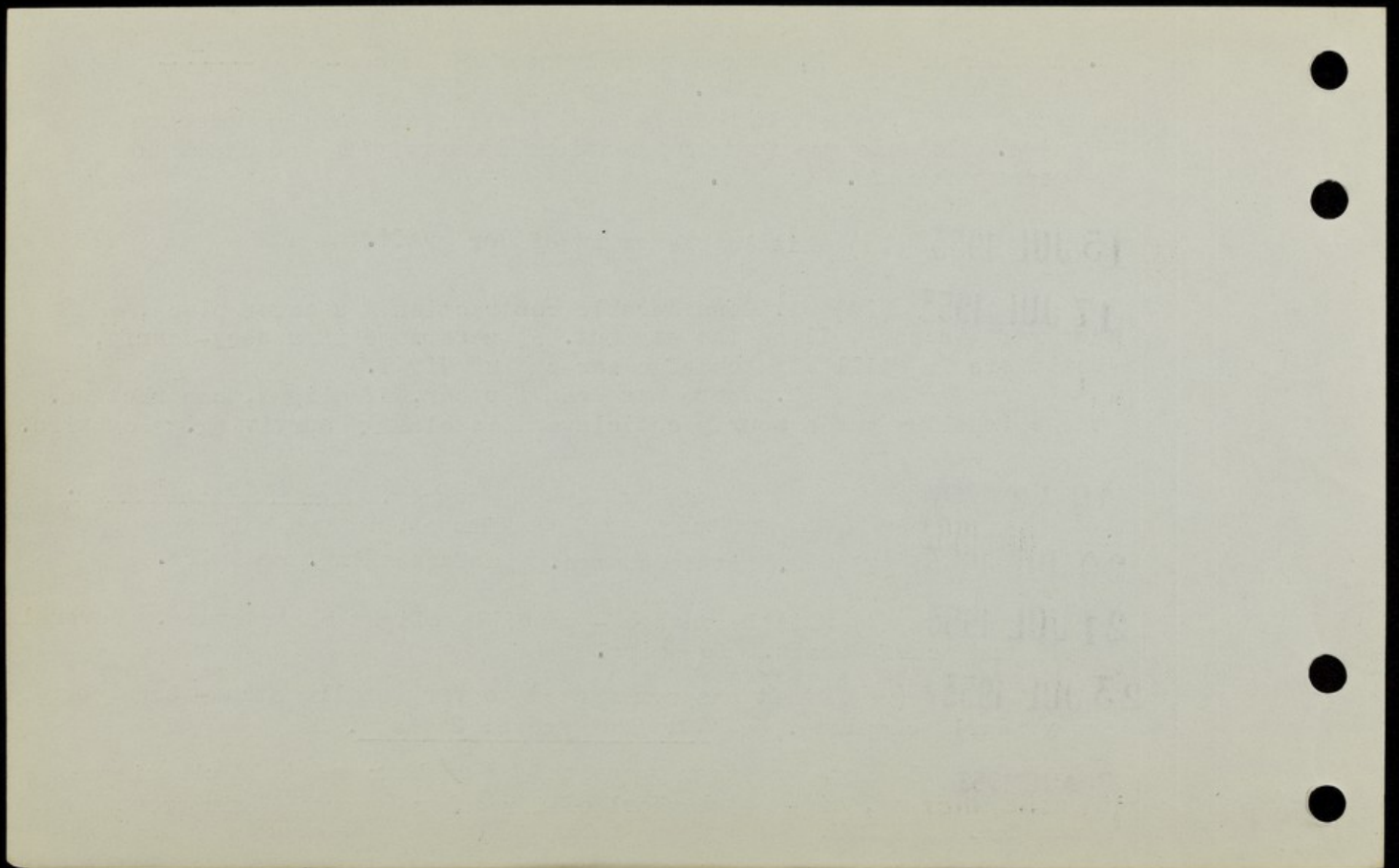
2. Graft is still hanging on, but no hairs.

20 JUL 1953 (23) 2. Little change. Surface shiny. no hairs.

21 JUL 1953 (24) Little change - possibly slight contracture. Several small and scurfy scabs. No hairs.

23 JUL 1953 (26) Graft has contracted to very small patch - breakdown very nearly complete. Survival score: 26 days.

7 AUG 1953 (41) Both grafts represented by small round bald clearings in skin of chest, with usual whorl of irregular (agouti) hairs round perimeter. DESTROY.



INOCULATION OF FOETAL MICE WITH ADULT CELL SUSPENSION
THROUGH BODY-WALL.

EMB-87

15 APR 1953 Donor tissue. Kill male of litter 4A3/4, b.5 March, remove aseptically $\frac{1}{2}$ spleen, $\frac{1}{2}$ kidney, one testis and a small piece of liver. Chop up finely with coarse scissors, suck through fine No.26 Agla needle, spin, resuspend in small volume of fluid (few drops of Ringer). Suspension exceedingly dense and uniform and contains masses of cells and small clumps.

Recipients. Litter of female (o-parous) from litter 4C4/2, b.2 Feb., pregnant by 4C3/1 male, special diet since 6 April.

5 approx. 17 days old foetuses seen, only 4 injected because of poor visualisation. 0.01 cc each. Injections probably intraembryonic and on the whole satisfactory.

Nembutal anaesthesia, recovery O.K.

18 APR 1953 Litter of 2 born overnight. Age at time of injection therefore 16 days or slightly more.

13 JUN 1953 Test-operation.

Donor: A-line male from Stock 1 (also used as donor to EMB-112). Remove 2 standard body-skin pinch-grafts, trim off panniculus, and store temporarily on Ringer-moistened filter-paper.

Recipients:

1. M 22 g. (R)
2. M 22 g. (L)

24 JUN 1953 (11)

1. Graft in advanced phase of breakdown, but a few islets of surviving epithelium remain. Do not rebandage.
2. Graft predominantly viable, but dorsally there is a largish area of scarred ulceration, whilst ventrally a small marginal scar is visible. The rest of the graft looks relatively healthy, but prognosis unceratin. Do not rebandage, germolene.

25 JUN 1953 (12)

1. Graft has scabbed - total breakdown. Kill.
2. Graft predominantly surviving, but epithelium looks rather delicate. No sign of follicular activity.

27 JUN 1953 (14)

1. Contracture has taken place, and the graft is obviously going through a reaction and is likely to be on the way out. The epithelium is still delicate and whitish, no sign of hair-growth or even follicular activity, and parts of the graft are beginning to scab.

28 JUN 1953 (15)

1. Scabbing is almost complete. Survival score is therefore about 15 days. i.e. short-term prolongation.

INTRAEMBRYONIC INOCULATION OF FOETAL MICE (cba to A)
WITH ADULT CELL SUSPENSION THROUGH BODY WALL.

EMB-77

10 FEB 1953 Donor tissue: Kill Male of litter 3C1/3, b.28 Oct., and remove 1 testis, a very small piece of kidney, a very small piece of liver, and 2/3 spleen. Chop up with scissors and prepare very dense suspension (almost brei) in Ringer. Inject each foetus with 0.01 cc.

Recipients: Foetuses of A-line female of litter 4A3/1, b.15 Nov., 35g., pregnant by litter-mate. Supplementary diet since 17 Dec.

3+2 foetuses seen and injected with 0.01 cc. Very good visualisation and ops. satisfactory.

Embryos about 17 days old.

12 FEB 1953 Litter of 7, but 2 dead shortly after birth.

13 FEB 1953 4 healthy-looking survivors. 4

14 APR 1953 Test operation.

Donor: ex-2C3/6 female, b.2 Feb. Remove standard pinch body-skin grafts, scrape off panniculus, and transplant in usual way to scissor-clipped beds.

1. M (no mark) 27g.

2. F (R) 21 g.

3. F (L) 22 g.

4. F (no mark) 22 g.

All ops. satisfactory.

22 APR 1953 (8)

1. Healing faultless. Pull off cuticle off which tends to adhere, therefore leave small patch in position. Roof healthy pink, faint trace of pigmentation, no sign whatever of a reaction. Hopeful. Rebandage.
2. Healing faultless. Pull off cuticle which tends to adhere. Roof slightly moist but healthy pink and fully epithelialised. Only sign of a possible reaction is a deep pink patch postero-ventrally which becomes more prominent on exposure to air. Rebandage.
3. Healing faultless. Cuticle tends to adhere - pull off. Widespread points of haemorrhage and weakness - specific reaction has commenced though survival is as yet very good. Rebandage.
4. Healing faultless. Cuticle off cleanly with tulle. Roof moist and rather glistening, and faint signs of vascular inflammation. But as yet complete survival. Rebandage.

25 APR 1953 (11)

1. Graft uncontracted and has an apparently dry roof which peels off to reveal moist dermis with a slightly yellow tinge. Scabbing on exposure. Kill.
2. As for 4 but pigmentation less pronounced. Germolene. Supermouse?
3. Graft looks superficially pretty good but epidermis tends to peel. There is a just perceptible blue colouration. Rebandage.
4. Graft soft and swollen (domed) with firm, dry roof and deep leaden blue pigmentation. Graft skin obviously active. Scaly cuticle. Super-mouse?

2.
27 APR 1953 (13)

- 2. Graft in position and fully surviving. Posterior 2/3 well pigmented (but less than 4), and this part of graft is thick and slightly domed. The anterior 1/3 is pink and thin, and has a very small and probably non-specific scar. Border-line of pigmented area very incisive. Very short hairs are just visible. Supermouse.
- 3. Typical breakdown. Kill.
- 4. Graft thick and domed, very deeply pigmented, and growing agouti hairs all over its surface. Supermouse.

1 MAY (17)

~~~~~  
These animals were photographed yesterday. (Calova) 2.7 ✓  
4. ✓  
2.4. ✓

- 2. Has grown thick fur in posterior 2/3, but anterior region is rather inflamed and its margin feebly scabby. The graft is undoubtedly going through a mild reaction, and has probably done so all along since the anterior 1/3 had never quite settled down ~~at~~ to produce a satisfactory hair crop. The small scar noted very early on was probably a symptom of that reaction.
- 4. Graft in excellent shape and completely covered by thick pelt of agouti hairs - also very supple.

4 MAY 1953 (20)

- 2. The anterior scabbing has become more prominent~~ly~~ and the graft has lost its suppleness (now palpable as a whole). The hair-growing area is also somewhat reduced in size; whilst it is clearly still surviving prognosis is now poor. Photograph (Calova) ✓

4. Graft supple and without sign of a reaction.

5 MAY 1953 (21)

2. The scab has become more extensive, with corresponding decrease in the hair-bearing area. Yet a small patch (centro-posteriorly) remains carrying agouti hairs and with healthy-looking pigmented epidermis. But graft undoubtedly on way out.

6 MAY 1953 (22)

~~2. The scab has become more extensive, with corresponding decrease in the hair-bearing area. Yet a small patch (centro-posteriorly) remains carrying agouti hairs and with healthy-looking pigmented epidermis. But graft undoubtedly on way out.~~

4. Quite unchanged.
2. Decrease in area of hair-bearing region continuing.

8 MAY 1953 (24) EMB 77.4 photographed yesterday in colour. Found dead this a.m. (Subcutaneous nembutal + ~~supplementary~~ supplementary ether + cardiazol.) After clipping corpse, rephotograph in b/w: this graft was impeccable in every possible respect. Treat as below.

77.2. Clip. In spite of, uch contracture, there is still a tiny island of smoky-looking epithelium (representing the active area) where there may still be graft survival.

TEST OPERATIONS ON 77.4

Close clip graft and field; wash with soap and water; no spirit. Then excise two rectangles, each comprising one half of the graft +

area of host albino skin. Transplant one to an albino, viz: male of litter 5A3/4, b.4 April, wt. 20 g., and the other to a CBA mouse, viz: male of litter 5C2/2, b.21 March, wt. 22 g., no ear mark. The graft on the CBA mouse was much too thick for the bed. Excellent operations in both cases: the grafts were of course well trimmed before transplantation, but both were thick.

Orientations:



On albino recipient



On CBA recipient

NOTE: 7.30 p.m. same day: cba recipient dead, all attempts at revival having failed. Remove graft from corpse (graft clean and in good shape) and transplant to male of litter 2C3/4, b.31 August, r.anterior notch. Orientation exactly as described above. Keep animal in cage of its own.

**16 MAY 1953** 8-day inspection.

Cba recip. Cuticle peels. Healing very sound. Graft as a whole rather

15 MAY 1953 (31) EMB-77.2. There is now no definite trace of any surviving CBA epithelium on this mouse. Thus on this mouse there is evidence of an exceedingly weak and prolonged reaction, clearly in evidence by 17th day (possibly by 13th), and complete shortly after 24th day.

**16 MAY 1953** 8-day inspection.

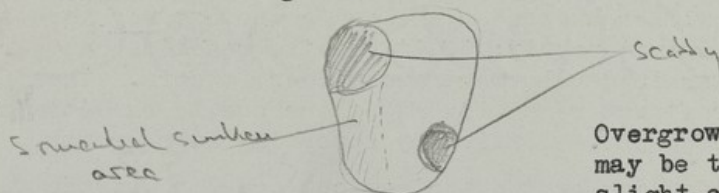
Cba recip. Cuticle peels. Healing very sound. Graft as whole rather angry-looking, dark pinkish, and epithelialised. Not much to choose between the two components at this stage. Rebandage.

A-line recip. Cuticle off with tulle, graft soundly healed and much as on the  $cb_a$  r recip. Rather dark pink. Rebandage.

**18 MAY 1953** (24 + 10) Inspectionx of A-line recip (at MST for CBA graft). No obvious dividing line; the graft as a whole, except for antero-dorsal quarter, has a rather angry-red looking appearance. There was a raised-up inflammatory patch antero-ventrally (i.e. in the albino part), and on picking at the central epithelium a small defect was created which bled. There was also a definite patch of weakness postero-dorsally, i.e. in the CBA part of the graft, and the epithelium peeled away from here to leave a small ulcer. But there is quite certainly surviving epithelium in the CBA part generally. Thus the reaction of the CBA part seems delayed, and the albino-part seems to have elicited anyhow a minor reaction. (Note that the graft was taken post mortem with panniculus, & had to be radically trimmed.)

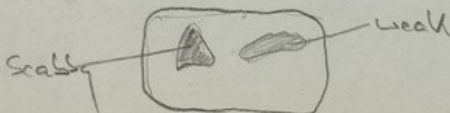
19 MAY 1953 (24 + 11)

A-line recip.: Still no very clear-cut picture as to what is going on. Graft as a whole is pink and epithelialised with the exception of 2 scabby areas, the larger of which is certainly in cba territory. But there is a suspicion that the posterior part is a little sunken compared with the anterior region. On the whole still unconvulsive.



Overgrowth of the cba component may be taking place. There has been slight contracture.

Cba recip.: Clear distinction between dorsal and ventral halves, but **NOT** as would be expected from the original graft diagram!  
 Ventral half: a little raised and pink, but epithelium sound.  
 Dorsal half: posteriorly with definite ulceration, anteriorly with definite weakness but clear survival.



It is to be assumed (?) that a mistake was made either in the presentation of the original diagram or in the orientation of the graft at time of operation. Germolene, no bandage.

22 MAY 1953 (24 + 14)

**Albino recip.:** Since the last inspection no clear-cut breakdown or scabbing of the cba component has been observed. The small scabs previously noted have now been shed and only two very small scars remain just within the dorsal half of the graft. The rest of the graft looks healthy pink and is soundly epithelialised. There has undoubtedly been considerable contracture.  
**Conclusion:** breakdown of the cba component may have taken place slowly and insiduously; if it has the reaction has certainly been slower in development and milder in form. That portion of the graft now remaining may therefore be expected to be the A-line component. Confirm by observing hair-growth.

**Cba recip.:** During the last day or two a clear-cut scabbing of the ventral half of the graft has taken place, whilst the dorsal half is soundly epithelialised and looking very healthy.  
**Conclusion:** The orientation of the graft is after all as shown in the original diagram. The ventral half (**A-line**) has broken down a little more slowly than would be expected, whilst the dorsal component (**cba**) was subjected to a mild reaction but has now settled down to an auto-graft-like existence. Confirm by observing hair-growth.

General note: in this experiment there has been some difficulty in distinguishing the early behaviour of the two components; this is due to the fact that the cba component was not active when transplanted back. If the above conclusions are correct then this experiment does not differ significantly from EMB-62.

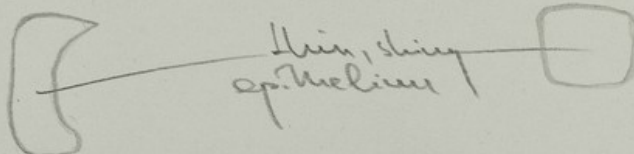
28 MAY 1953 (24 + 20)

EMB-77

Neither of the two graft && remnants on the albino and cba recip. show any sign of hair-growth, and the areas look very much like resurfaced scars. It is doubtful whether a great deal of information will be gleaned from this experiment, but keep animals and inspect again in a week's time.

A-line recip

cba recip.



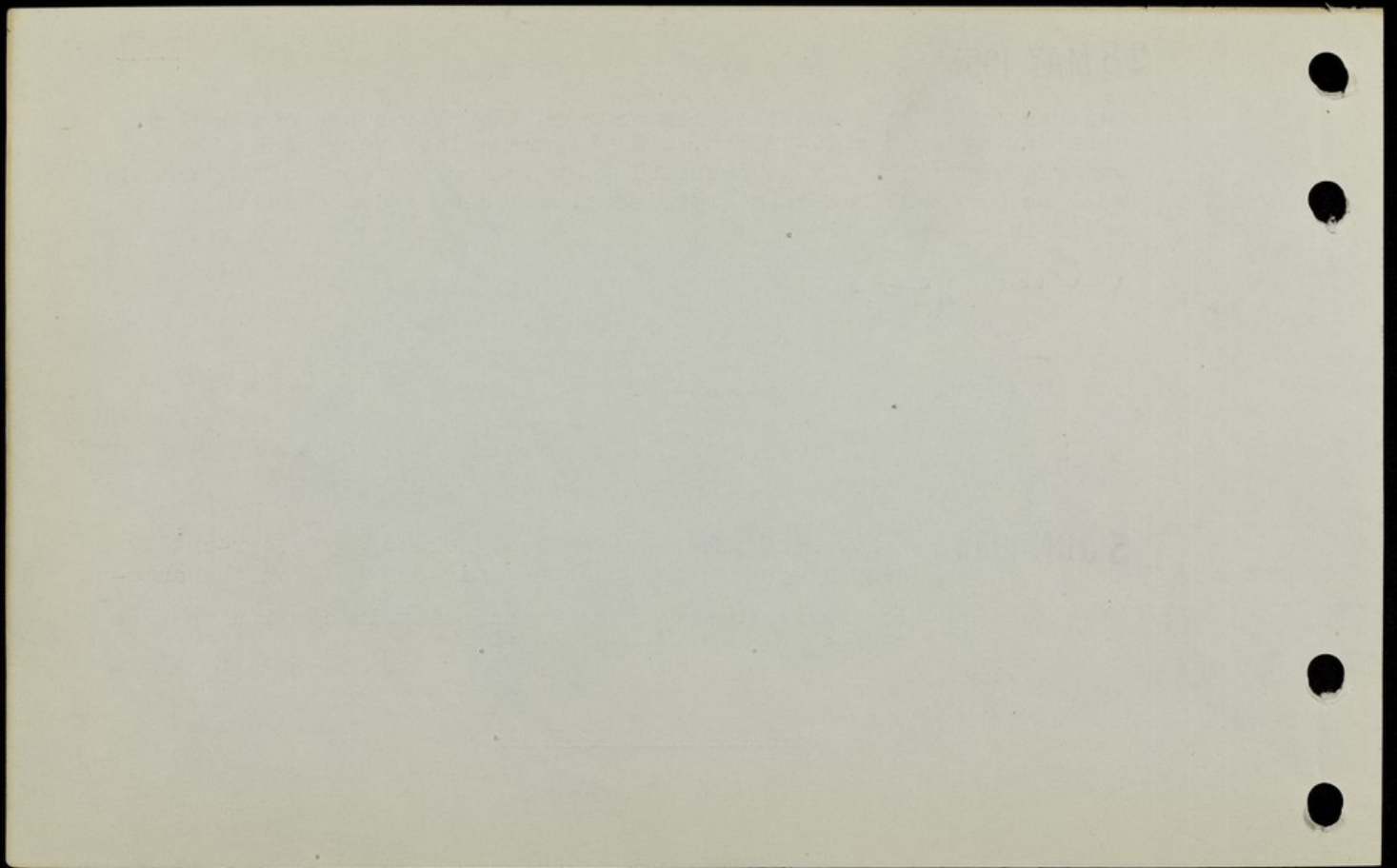
5 JUN 1953

Neither of these grafts has yielded any clear-cut result - there has been no hair-growth from the auto-logous areas and there is no point in continuing the experiment. Destroy animals.

Experiment concluded.

53

5 JUN 1953





**30 JAN 1953**

Donor tissue: Exactly as for EMB73.

Recipients: Near term foetuses of 3C5/P, being its 5th litter.  
Mother 46 g. and b. 22 July. Supplementary diet since operation  
but not before.

Ops exactly the same as for EMB-73. Visualisation however was very bad  
and injections not too satisfactory. 5 foetuses were injected and it  
is probable that this represents the whole litter. 2 injections rather  
blind.

**31 JAN 1953**

Litter born 1030 a.m. One mauled and dead shortly  
after, but 7 healthy survivors remain.

**31 MAR 1953**

Test operation:

Donor: Female (early pregnant) of litter 3A4/3, b.10 Sept. Remove  
standard body-skin pinch grafts, trim, and transplant to standard  
scissor-clipped bed on RH S.

Recipients:

1. F (R) 22 g.
2. F (L) 20 g.
3. F (no) 19 g.

4. M (R) 19 g.
5. M (L) 22 g.
6. M (no) 22 g.
7. M (both) 22 g.

**11 APR 1953** (11 - MST)

1. Cuticle off cleanly as dry scab. Graft slightly swollen, moist, very angry pink with haemorrhages and mild erosion, Violent reaction. Scabbing on exposure is proof of breakdown complete. Kill.
2. Cuticle tends to adhere, peel off carefully. Roof dry and epithelialised, healthy pink. Possible flaw: slight unevenness of surface. High degree if not total survival. Super-mouse?? Rebandage.
3. Cuticle off cleanly to reveal moist roof with partial loss of epidermal surface. Scabbing. Kill.
4. Same as for 1. Kill.
5. Cuticle off cleanly. Roof dry, firmly epithelialised, pink, dermal pattern distinct, but slight weakness in centre. Survival very considerable if not complete. Super-mouse? Rebandage.
6. Typical breakdown. Roof very moist and blotchy, no survival. Kill.
7. As for 6. Kill.

**13 APR 1953** (13)

2. Graft uncontracted, dry roof, obviously epithelialised, scurfy cuticle, uniform but rather strongly pink, otherwise no sign of a reaction. No hairs.
5. Graft uncontracted and epithelialised, covered by thin scaly cuticle. Weak spot noted last time covered by adherent scaly cuticular scab.

2.

Colouration rather too pink. Dermal pattern has disappeared. <sup>EMB-74</sup>  
Surface not mat. Survival probably nearly 100% but graft going  
through a sticky period. Prognosis rather uncertain. Germolene.

~~12 APR 1953 (13)~~ [~~Probably 14 April = 14~~] 15 APRIL = 15

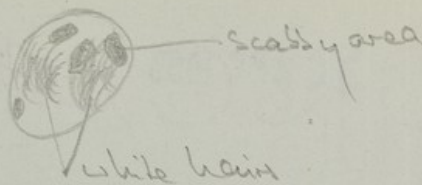
2. Still no significant contraction. Colour now healthy white with slight tinge of pinkness. Thick scaly cuticle. No hair-growth. Obviously 100% survival, and graft has gone ~~to~~ through a mild reaction from which it appears to be recovering.
5. On the 13th a small haemorrhagic patch was seen- this gradually darkened and was clearly indicative of a reaction. Yesterday this patch ~~was~~ fairly large, oval, in centre of graft, there has been some contraction, but some surviving epithelium ~~is~~ clearly still present marginally. (This ~~is~~ not ingrowth). To-day scabbing has been completed and breakdown is complete. Kill. Breakdown of 5 therefore took about 14 <sup>15</sup> days, i.e. some prolongation was achieved.

16 APR 1953 (16)

2. Graft much as above, but hair-growth has commenced in two places. Recovery is certainly taking place.

18 APR 1953 (18) Hair-growth is taking place, but only at 2 points and very sparsely. The hairs are white and the graft is certainly completely viable; nevertheless the surface is still somewhat glistening and does not seem to have settled down completely. This suggests the continuation of a very mild reaction.

**21 APR 1953** (24) Yesterday some very slight marginal scabbing was noted and assumed to be due to non-specific causes. But to-day the scabbing has become very much more extensive and the general appearance of the graft leaves no doubt that it is going through a sticky period of specific reaction, and is very likely to succumb in the next day or so. There has also been some contraction, and the graft has lost its suppleness.



Colour-photograph.

**22 APR 1953** (22) The dorsal scabbing has become slightly more pronounced but on the whole the graft seems to be very nearly holding its own. Ventrally the epithelium is clearly viable, and it is an interesting fact that the reaction is exceedingly mild. Prognosis uncertain.

**24 APR 1953** (24) Scabbing and contraction has continued quite steadily and it is unlikely that there are many surviving cells. Breakdown-time of 24 days will not be far out.

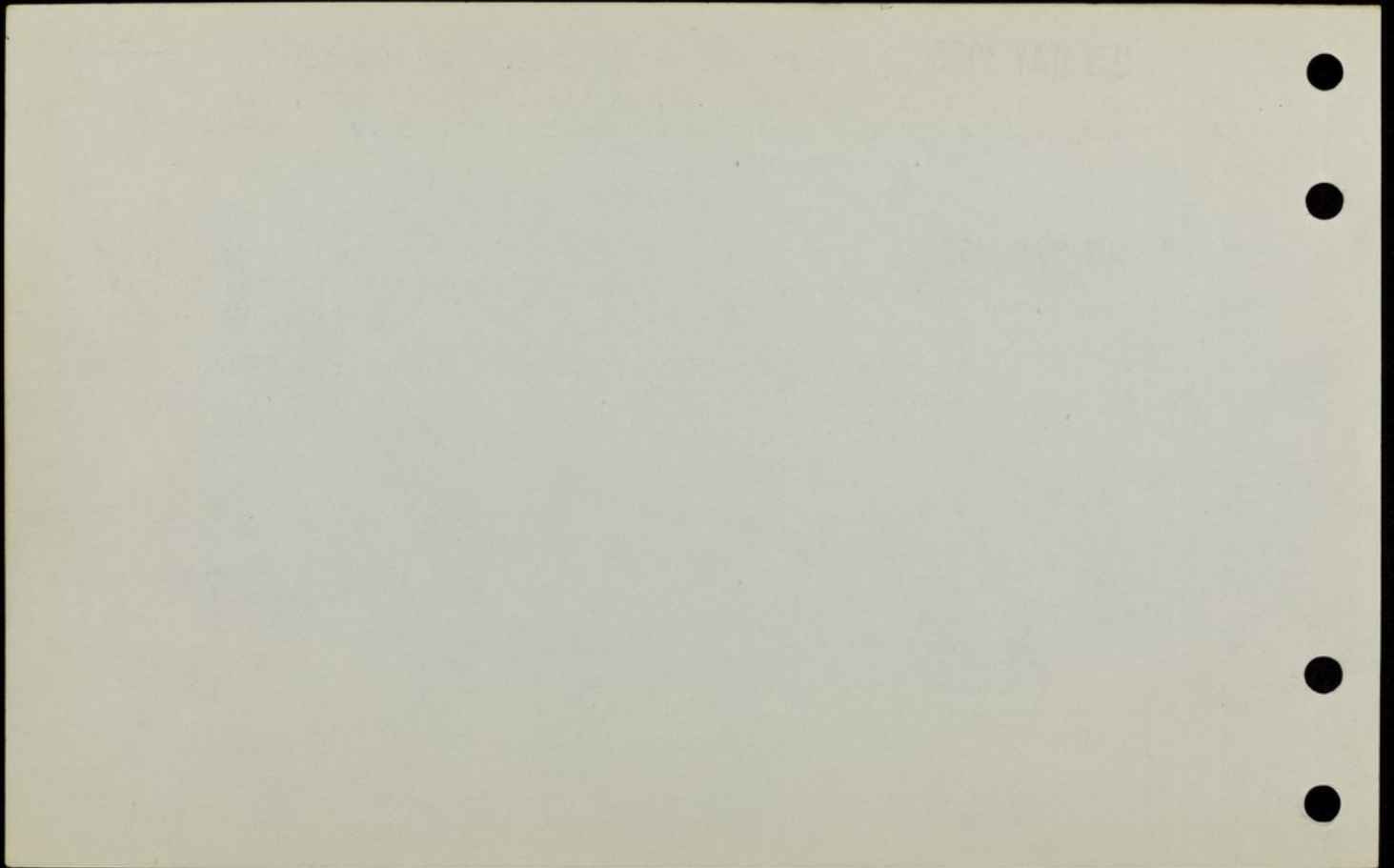
**19 MAY 1953** Reoperate: Graft from male of litter 4A4/2, b.17 Jan. LHS operation, usual scissors-prepared bed. Inspect at 6 days.

EMB-74

25 MAY 1953 (6) graft has well healed. Definite little patches of epithelial weakness, particularly in centre, but unquestionably some surviving epithelium, and hence **no clear-cut immune effect**. Rebandage.

27 MAY 1953 (8) The graft now shows signs of advanced breakdown, not impossibly complete. Cuticle and epidermis came away together to reveal damp and somewhat macerated and blotchy dermal surface. **Destroy**.

**NOTE:** Roughly speaking this represents an acceleration of breakdown from 24 to 8 days.



INTRA-EMBRYONIC INOCULATION OF FOETAL MICE (A to cba)  
WITH ADULT CELL SUSPENSION. THROUGH BODY WALL.

EMB-73

30 JAN 1953

Donor tissue: Kill 4A4/1 M., b. 24 Dec. and remove both testes, whole spleen and half of one kidney. Chop up fine and prepare dense suspension (brei) as for EMB-69. Inject 0.01 cc per foetus.

Recipients: 15-16 days old foetuses representing the first litter of a female from litter 2C2/6, b. 12 Nov. and pregnant by 3C4/3. Usual supplementary diet since 17 Dec.

Open skin in mid-ventral line and mobilise. 4+2 foetuses seen through body wall and all were injected. This probably represents the whole litter. Visualisation on the whole very good and injections definitely intra-embryonic (with the possible exception of one) and satisfactory. Body wall kept moist throughout.

Skin prepared by shaving and germolene, no spirit. Multiple sutures, each touched up with a dab of New-skin. Anaesthesia O.K.

FEB  
3 JAN 1953

Healthy litter of 5 born early morning - no sign of 6th. Age of litter at time of injection therefore c. 16 days.

**31 MAR 1953** Test operation.

Donor: Male of litter 4A4/3, b.12 Feb. Remove standard body-skin pinch grafts and trim as usual. Graft beds prepared by scissor-clipping.

Recipients:

1. F (R) 21 g. Snick in skin of RHS - stitch. LHS.
2. F (L) 22 g.
3. F (no) 21 g.
4. F (both) 22 g.
5. M (R) 25 g.

**11 APR 1953** (11 - MST)

1. Healing faultless. Graft flat, supple, with normal dermal pattern, only abnormality: surface not completely mat and a little on the transparent side; very slight suspicion of a patch of mild vascular inflammation. **Survival probably complete. Super-mouse?** Rebandage.
2. Epidermis in place but peels off easily. Scabbing of exposed dermis. Breakdown far advanced. Eventual scabbing. B.D. complete. **Kill.**
3. Graft flat and supple, no sign of a reaction, epidermis firm. **Complete survival. Super-mouse?** Germolene only.
4. Epidermis peels away easily revealing moist and somewhat haemorrhagic dermis. Graft puffy. Eventual scabbing on exposure. **Kill.**
5. **Completely auto-graft-like - flat, supple, firm epithelium. Super-mouse?** Germolene only.



2 MAY 1953 (33) All 3 grafts are doing very nicely - bushy hair growth and complete suppleness on palpation. Both females are pregnant, one has been segregated and they are being given the usual "special diet".

6 MAY 1953 (37)

3. This mouse has had a healthy litter of 6, pregnant by 5. All 3 grafts in excellent condition.

16 MAY 1953 (47)

1. Litter of 8 born this morning - pregnant by 5. All 3 grafts O.K.

19 MAY 1953 (50) Both litters doing well. All 3 grafts in good condition.

Reoperate 5., this time R<sub>HS</sub>. Graft from male of litter 4A4/2, b.17 Jan. Preparation of graft and bed by usual method. Opn. O.K. Inspect in 11 days time (MST). Segregate male from its litter-mates.

Note: a very small patch of white hairs was found on the LHS thorax, also sign of a very old scar. Could this be the point of injection?

20 MAY 1953 (51) It has been discovered that No.1 (the suckling female) was operated yester-day - not the male. Let the female therefore suckle half her litter (4), and farm out the other half to a suckling B-line

2.

**13 APR 1953 (13)**

EMB-1

1. Graft uncontracted, healthy colour, dry roof, incipient white hair-growth round dorsal margin, but otherwise as yet bare. Only flaw, which might well be indicative of a mild reaction, are 2 very small reddened patches. Germolene.
3. Graft perfect, uncontracted, healthy and growing white hairs over most of its surface.
5. A&S for 3, but hairs very short and present only on anterior half.

**15 APR 1953 (15)**

1. There have been unquestionable signs of a very mild reaction in the last 2 days - surface tending to glisten somewhat, slight reddening at one point, and retarded hair-growth. But graft has almost completely recovered, colour now healthy, and hair-growth has spread over most of it. Anterior 1/4 still bare.
3. Graft perfect. Good crop of white hairs all over graft.
5. Same as for 3.

Note: These 3 mice were put together into the same cage on the 13th so that mating will be taking place.

**21 APR 1953 (21)**

All 3 grafts are in perfect condition and continuing to grow a very dense and bushy fur.  
Colour-photograph No.3.

female whose own litter is sacrificed.

EMB-73

**22 MAY 1953** (52) No.1's litter is being suckled successfully both by the mother and foster-mother.

**30 MAY 1953** (50 + 11)

Inspect secondary graft on BHS: graft in excellent shape and without the slightest blemish. Don't rebandage. Litter being suckled very well both by mother and foster-mother.

BHS graft also in good shape. The same goes for 3 and 5.

**1 JUN** (50 + 13)

1. Secondary graft on RHS in excellent shape and appears to have settled down completely.

**3 JUN 1953** (50 + 15)

1. RHS graft now beginning to grow hairs.

**15 JUN 1953** (77) PASSIVE IMMUNIZATION OF MOUSE 73.5 (M).

Remove both the deep and the superficial axillary node from the operated side of each of two mice of series PT-3 (q.v.), making 4 (obviously hypertrophied) nodes in all. These mice had received standard RHS A-upon-CBA grafts 11 days ago (MST), and the grafts had broken down completely.

Clip the hairs from the A-line graft on EMB 73.5: this graft is faultless

condition in every respect, with dense even hair crop. Then chop the nodes into 30-40 pieces cleanly with cataract knives, load into standard tumour-implantation trochar, and inoculate intra-peritoneally under ether.

**Prognosis:** the graft is expected to show inflammatory & other degenerative changes. Repeat in 2-3 days' time if necessary.

(**IMPORTANT NOTE:** It was originally intended to immunize 73.3, viz: the female with only the single original graft; but, on clipping, this graft proved to be rather hard and to have a tiny but possibly significant central defect. **Watch.** It is just possible that a minor reaction is in progress. Cf. also day 15 (15 APR). )

**17 JUN 1953** Passive immunisation of mouse 73.5. cont.

Repeat exactly the same procedure as on the 15th: transplant intraperitoneally 2 superficial and 2 deep lymph nodes taken from the right axillae of two mice ~~bearing~~ (cba) bearing A-line skin grafts on their RHS thorax. The grafts, now 13 days old, are completely scabby; animals used are from PT-3. The lymph nodes were chopped into 40 pieces and inoculated intraperitoneally with standard tumour-implantation trochar; no anaesthesia.

**NOTE:** the graft on 73.5 looks in good condition and bears no clear-cut signs of a specific reaction, but it is just possible that there is a very faint reddening of its surface. Graft about 7mm by 8mm.

**73.3.** This graft is clearly passing through a phase of a highly modified reaction. It is still palpable and the central defect noted

two days ago is now very much more marginal: this seems to indicate that the graft is contracting. Watch carefully.

73.1. Both the RHS and LHS grafts are supple and without any sign of a reaction; both are well covered with albino hairs.

**18 JUN 1953** 73.5. The faint reddening noted yesterday (2 days after transplantation of node material) has become slightly more intense and there can be little doubt that the graft is beginning to experience a frightfully mild reaction. There is no sign whatever of epidermal breakdown, and the short hairstumps left after clipping resist attempts to pull out with forceps.

**19 JUN 1953** 73.5. (4th day). The reddening, which is presumably indicative of vasodilatation and possibly minor haemorrhage, has become a little more pronounced and it is now possible to see it clearly enough without having to stretch one's imagination in the least! It is also rather patchy. But so far no signs of epithelial breakdown; hair-stumps still firmly anchored to their roots.

**20 JUN 1953** 73.5. (5th day). No visible change. Graft slightly orangy in colour, with patches a little darker than the rest. Hair stumps firmly anchored.

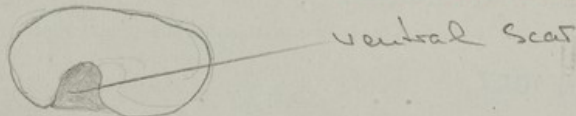
73.1. Graft continuing to pass through a mild reaction which is producing considerable contracture; this is most probably occurring in the region of the scar which is almost completely

maeginal, whereas it originated as a central defect. The rest of the graft surface looks reasonably healthy, but the dorsal margin too seems to be slightly affected and scurfy.

73.1. Both grafts are in good condition and entirely supple.

23 JUN 1953 (85) (77 + 8)

73.3. Graft still further contracted, and now consists of dorsal horse-shoe of pretty healthy-looking epithelium (white and with hair-stumps) surrounding a ventral scar. This ventral scar originated as central blemish when reaction was first noticed. COLOUR-PHOTOGRAPH.

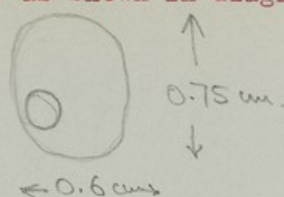


73.1. Both grafts in good shape, with bushy hair crop, and very supple. Colour-photograph, first both together with animal seen from above, then the original graft on LHS.

73.5. Graft has probably contracted slightly, is perceptibly palpable (having lost its suppleness) and somewhat swollen. The haemorrhagic pattern has not changed a great deal since last time, but there has been slight intensification and parts of the graft epithelium tends to be shiny. COLOUR-PHOTOGRAPH.

In order to discover whether there has in fact been a lymphocytic reaction, and whether the epithelium has been attacked in any way, remove small biopsy-specimen (as a pinch) from ventro-

posterior corner as shown in diagram. Fix in Formol/Hg.



(77+9)

**24 JUN 1953** Graft has contracted still further, but this is to some extent at least due to && removal of biopsy specimen. But graft is generally in poor condition, rather red and shiny, sufficiently swollen to be palpable (contrast its great suppleness at time of immunisation) and may well not stay the pace. Note that the removal of the very small biopsy spec. has hardly interfered with the graft.

<sup>8</sup>  
**27 JUN 1953** (77 + 13)

3. Contraction has advanced still further, leaving a small crescent of viable epithelium bearing albino hairs. It is quite clear now that the reaction which has been mild and long drawn out will go to completion.
4. Both grafts in good shape. This mouse has had another litter. (Mated with 5).
5. Graft has contracted a great deal over the last few days, and the reaction which has been in progress for some time now has probably

come to completion. The graft is grossly discoloured (yellowish-brown) and almost scabby - it is extremely doubtful whether it has any surviving epithelium. Its rims are beginning to loosen and undermining is taking place. COLOUR-PHOTOGRAPH.

BREAKDOWN OF THIS GRAFT HAS THEREFORE OCCURRED ~~XXXXXXXX~~ 11-13 DAYS AFTER THE FIRST TRANSPLANTATION OF IMMUNE LYMPH NODES. THE FIRST SIGN OF A REACTION WAS OBSERVED 2-3 DAYS FOLLOWING TRANSPLANTATION.

NOTE THAT THE ABOVE TIMINGS ARE BOUND TO BE INACCURATE, BUT THE HISTOLOGICAL REPORT ON THE 9-DAY BIOPSY MIGHT HELP TO CONFIRM THEM.

**29 JUN 1953** (77 + 14)

3. Only a tiny islet of epithelium bearing a few white hairs remains, and survival is sufficiently doubtful even in ~~this~~ that breakdown can now be said to be complete. But keep animal and transplant another A-line graft in a week or two so that the absence of immunity can be firmly established.

SURVIVAL-TIME OF EMB-73.1.: 91 days.

5. Graft grossly discoloured and looking very scabby, rims free and advanced undermining is taking place so that the graft could be pulled off with the greatest of ease. This confirms the above estimate of the graft's survival time. Keep animal and transplant yet another A-line graft in about 4 weeks time in order to determine the duration of the passive immunity.



**27 JUN 1953** Test-operation on litter of 3.

Litter about 7 weeks old.

Donor: Standard grafts from female of litter 4A2/3, b.12 Jan.

Recipients:

- 1. (R) M 24 g.
- 2. (L) M 24 g.
- 3. (no) F 20 g.
- 4. (both) F 20 g.
- 5. (2R) F 22 g.
- 6. (2RLL) F 20 g.

**8 JUL 1953** (11 - MST)

The six grafts look like a typical control series. 1 shows a fairly high degree of survival, 2 are certainly total breakdowns, and 3 show advanced reactions - possibly complete.

Retain these animals for passive immunisation of EMB-73.3.

**9 JUL 1953** PASSIVE IMMUNIZATION ETC. of MOUSE 73.1.

Anaesthetize with Nembutal & close-clip both grafts. The original = LHS graft, now 101 days old, is delicate pink in colour, densely haired, of firm consistency and slightly thicker than host skin. A perfect graft. Area  $10\frac{1}{2}$  mm AP X  $9\frac{1}{2}$  mm DV in relaxation. The second stage = RHS graft is of 51 days standing: this graft is thin and somewhat transparent, and main lateral blood vessels could be seen through it. The hair growth also rather thin: probably belly or ventro-lateral skin.

(A) Excise the 51-day RBS graft as a pinch including panniculus: repair donor area with 4 sutures: only one small sector of rim of graft left behind. Trim and transplant to CBA female, wt. 22 g., from Stock II: usual procedure.

(B) Kill three mice from the EMB-73 litter homografted on 27 JUNE (see report on 8 JULY): the homografts of 12 days standing are now scabbed. Remove the superficial and deep axillary nodes from the operated side of each of the three (obvious hypertrophy) and chop each into 10-15 pieces.

Load the equivalent of 4-5 nodes into trochar and implant intraperitoneally into EMB-73.1. Then reload with the residue, representing 1-2 nodes, and implant under the integument in mid dorsal line. In both cases, massage to distribute the node fragments.

To be repeated in two days time.

**11 JUL 1953** (101 + 2) Passive immunisation cont.

73.1.: Repeat exactly the same immunising procedure as used on the 9th, but this time merely reinforce with 2 sets of 2 <sup>nodes</sup> grafts each. Transfer intraperitoneally, and inject tissue juice left behind after chopping up of lymph nodes subcutaneously.

So far the graft on this animal shows no visible signs of a reaction.

**13 JUL 1953** (101 + 4)

1. First sign of a possible reaction - a small reddened patch more

or less in centre of graft. Otherwise it is supple and O.K. EMB-73

**14 JUL 1953** (101 + 5)

1. Symptoms of ~~reaction~~ reaction now unmistakable though as yet very mild - the patch of reddening has become more intense and must be indicative of vascular inflammation. A faint orangy tinge may also be spreading over graft which is as yet pretty supple. **But note contracture:**  
D-V 0.80 mm (cf. 0.95)  
A-P 0.95 mm (cf. 1.05)

These measurements are admittedly roughish since the animal was not put down, but they are unlikely to err on the wrong side.

Note that these observations are in complete accord with those of No. 5.

**16 JUL 1953** (101 + 7).

1. Patch of reddening noted at +5 has become very angry & livid in colour. Elsewhere definite orange-yellow tinge. Reaction proceeding.

**16 JUL 1953** (61 days). TEST OP. ON FIRST LITTER FROM 73.1 X 73.5 b.16 May.  
8 mice. Standard RHS opns using skin from Stock II A-line female in very late pregnancy.

(If negative, these animals to be used as primary hosts for P.T. experiments.)

**17 JUL 1953** (101 + 8). Further deterioration of the graft on 73.1 under passive immunization. Dimensions: AP X DV = 8 x  $7\frac{1}{2}$  mm.

**20 JUL 1953** ~~(101 + 11)~~ (101 + 11)

73.1. Dorsal patch of livid inflammation (noted since 4th day) has become scabby and (due to contracture of graft) almost marginal. Areas of severe and deep-seated inflammation have spread over large ~~and~~ parts of graft which now appears to be in a very bad way.

Note further contracture: D.V.: 7 mm. A.P.: 7 mm.

Inspection of cba recipient of ex73.1 supergraft. (11)

Clear-cut survival - probably 100%. Surface dry and epithelialised, but there are 3 patches (post., ant., and dorsal) of inflammation which on drying tend to scab over. Hence a reaction is in progress, but there is clearly some prolongation of survival. Germolene.

**21 JUL 1953** (101 + 12)

73.1. Much as yesterday - further deterioration but still some survival.

ex73.1 graft: Graft is scabbing and becoming card-boardly, but probably still some survival.

**22 JUL 1953** (101 + 13)

73.1. No further contracture. Graft very blotchy, hard and discoloured.

ex73.1. Scabbing complete. **Survival score 13 days** - i.e. 2 days prolongation. Use animal as primary host in PT 5+6.

**23 JUL 1953** (101 + 14)

Graft still hanging on, but there has been further deterioration and graft may soon be scabbing. Animal had 3rd litter yesterday - this pregnancy may be responsible for long drawn out reaction.

24 ~~24~~ JUL 1953 (101 + 15)

73.1. Further contracture: V.D. 5 mm. A.P. 5 mm.  
Scabbing in progress. Little survival.

25 JUL 1953 (10L + 16)

73.1. Graft is now a card-board scab. No survival likely.

Survival time of graft since first inoculation of immune lymph nodes therefore about 15 days.

27 JUL 1953 (101 + 18)

73.1. Graft has completely and utterly scabbed. Keep animal and test-operate again after the passive immunity has worn off.

Note that this animal is suckling its 3rd litter, whilst 73.3 has also had 2 litters (but has not been mated again).

M.S.T. inspection of 1st litter of Emb-73.1. (11)

All 8 mice in this litter have grafts which show very far advanced breakdown, and all grafts scab (completely or nearly so) after exposure. Actively acquired tolerance is clearly not transferable to the offsprings.

28 JUL 1953 REOPERATE WITH A-LINE GRAFTS BOTH 3 AND 5. Opns. LHS.

Donor is A-line male from stock 3. Operation on 3 not too satisfactory - bed exceedingly fatty (probably mammary tissue) and much bleeding.

*41 days after last dose of immune lymph nodes*

**3 AUG 1953 (126)**

(A) 6-day inspection of test-graft on 73.3 F. (The original graft on this mouse underwent 'spontaneous' involution, first seen at 77 days.)

Graft is in place. There is some epithelial weakness centrally which may be non-specific. Central pinkness. Certainly a high degree of epithelial survival. Replaster.

(B) 6-day inspection of test-graft on 73.5 M. (43 + 6 days after first implantation of lymph nodes to passively immunize against graft of 77 days' residence.)

Graft in place. Cuticle is adherent; graft has pink flush and definite signs of a vascular reaction... 'angry' appearance confirmed by candling. Certainly greater part of epithelium survives.

Replaster both and inspect at 8 days

**5 AUG 1953 (128)**

(A) 8 day inspection of 73.3 F. The graft has tiny scabs corresponding to the area of weakness noted at 6 days; these scabs are really little plaques of dried serous exudate, and the epithelium looks as if it had healed over underneath. The posterior  $\frac{2}{3}$  of the graft is firm, white and normal.

(B) 8 day inspection of 73.5 M. The entire cuticle came off in one sheet; the exposed surface was quite extraordinary in appearance, being

(EMB.73 cont)

uniformly scarlet, just tinged with blue, as if the whole graft were riddled with dilated vessels. On drying in air, the appearance was not quite typical; there was little contracture and the graft did not scab. It is therefore just possible that the exposed surface is epithelialized.

That there has been a reaction is conspicuously obvious; it may be purely a vascular reaction of the histamine-type, but this remains to be seen.

This animal breathed irregularly and fitfully under Nembutal: do not use again.

Replaster both and inspect at 11 days.

**8 AUG 1953** (131)

(A) 11-day inspection 73.3 F. The posterior  $\frac{2}{3}$  rds of the graft, which looked good at 8 days, now has a peeling epithelium; the anterior part is still fairly firm. Thus **breakdown in progress** but not yet complete. **To be followed up.**

(B) 11-day inspection of 73.5 M. The graft shows abject necrosis of very long standing, with secondary infection. Probably therefore breakdown was complete at 8 days.

This result very interesting, since it could not possibly be due to awakening of host's own resistance to graft in the interval between the first passive immunization and now. Had this animal at last developed

a 'spontaneous' resistance within this period, the reaction against the test graft could hardly have been so savage (cf. 73.3 female). It looks as if the implanted nodes had taken root and were giving a secondary response, i.e. as if this were a typical secondary response & not the consequence of passive transfer of antibodies. LOOK FOR THIS AT AUTOPSY AFTER ATTEMPTING A SURGICAL EXCISION OF PERITONEAL NODES?

10 AUG 1953 (133).

- A) 13 day inspection of 73.3 F Graft now looks like a desiccated, cratered and much contracted brwn scab. Not the slightest indication of survival.
- B) 13 day inspection of 23.5 M as above but there is a narrow marginal annulus of the graft collagen which looks as though it has been resurfaced with native epithelium.

26 AUG 1953 Reoperation of 73.1. (This animal had its A-line graft destroyed by passive transfer). (46 days plus last dose of immune nodes).  
Donor: standard graft from body of Stock 3 A-line female.  
RHS operation. Open fit.

26 AUG 1953 NOTE that 73.1 had its 3rd healthy litter on the 22nd July, and 73.3 its 2nd (7) on the 12th.



**1 SEP 1953** 73.1: 6-day inspection.

EMB-73

Healing satisfactory - slight bleeding dorsally due to somewhat careless removal of plaster. Cuticle rather adherent - peel off some of it to reveal dry, healthy pink and epithelialised surface. Graft also flat and supple, and is 100% surviving.

**Certainly no immune effect.**

**6 SEP 1953** 73.1 - 11-day inspection. The graft is slightly swollen, & unduly pink. Breakdown is in progress, but between the irregular areas of epidermal erosion there is still dry epithelium which is quite resistant to scraping.

**7 SEP 1953** (12 days) - Graft on 73.1 still shows approx 50% survival.

**8 SEP 1953** (13 days) Graft on 73.1 still shows fair survival dorsally - the rest is scabbed.

Reoperate 73.3 and 5. Since the breakdown of their original 'super-grafts' (3 spontaneously and 5 due to passive transfer) these animals have already been tested with another A-line graft, and both cases breakdown was prompt (see notes). In the case of 73.5 double the time between passive transfer and the first test has now been allowed to lapse (i.e 41 + 42 = 83 days).

Donor: 2 male A-line Stock 5 animals - take one graft from each and return to stock-pot. Usual operations - **RHS.**

**11 SEP 1953** (16) 73.1: the fate of this graft has been followed carefully from day to day, and it is pretty certain that the dorsal patch of surviving epithelium (noted at 13 days inspection) is still fully surviving. The rest of the graft has almost completely disappeared and the area which it had occupied has contracted ~~to~~ into a very small scar ventral to the patch of survival. It is impossible to be too dogmatic on the question of survival since the area is of course completely surrounded by host epithelium, but L.B. is fairly confident about it. If surviving graft epithelium is present then in any case ~~th~~is should reveal itself through hair-growth.

**14 SEP 1953** 73.1. A few white hairs indicate that the remaining graft area is ~~indeed~~ indeed graft epithelium. The passive immunity had therefore become very weak at time of transplantation of this test graft which ~~has~~ may have absorbed the remaining antibody. **Yet a further test-graft in a week or two will therefore be justified.**

73.3. Healing of this 6-day old graft is satisfactory - the anterior rim is free due to traction on removal of plaster. Cuticle very adherent, graft 100% viable though colour is very pale. Rebandage.

73.5. Healing satisfactory. Cuticle adherent. Graft surface is blotchy, with patches of epidermal weakness, but high degree of survival. Could just be a very mild immune effect. Rebandage and inspect again at 8 days.

**18 SEP 1953** (18) 73.3. B.D. is far advanced in several parts of graft, but there is still some survival - though not a great deal - centrally. Rebandage.

73.5 (8) Breakdown far advanced - epidermis pulls off as sheet. Some survival is however likely near the margins and in the anatomically intact epidermis. Rebandage.

25 SEP 1953 ~~73.1~~ 73.1. (30) A few white hairs still persist, but survival is very doubtful since the appearance of white hairs on the graft area may be non-specific. Only another graft can decide the state of reactivity of the host.

73.3. (15)



robustly epithelialised tyre

delicately epithelialised and rather pink area

small 'healing' scab

Diagnosis very difficult, though it would seem that there is some graft survival. Continue to watch.

73.5. (15)



scab

delicate epithelium

Could there be some surviving graft epithelium? The area does not look like a conventional scar and survival is just feasible.

Yet another graft will have to decide the issue.

5 OCT 1953 73.3 may have genuine survival, but

REOPERATE ALL ♂ MICE, this time on the LHS. Donor: male from 7A1/3. Inspect at 6 days. This transplantation should clinch the question of the recipients' reactivity.

11 SEP 1953 (6).

- 73.1 Graft has healed rather badly & has some desiccated overlaps, but certainly some surviving epithelium.
- 73.3 Graft certainly shows some surviving epithelium but weak in patches which ~~wild~~ dried off in air, and generally reddened.
- 73.5 Advanced breakdown, puckering and gross discoloration in air. Little evidence of surviving epithelium.

Leave unbandaged. 24 hrs later, only one graft showed any trace of surviving epithelium. In general, a strong immune effect in two, and a just perceptibly weaker effect in a third.

EXAMINATION OF NODES

- 1 and 5. There was no trace of the inoculated lymph node fragments in spite of prolonged search.

EXPERIMENT CONCLUDED.

INTRA-EMBRYONIC INOCULATION OF FOETAL MICE (aaUU to A)  
WITH ADULT CELL SUSPENSION. THROUGH BODY WALL.

EMB-69

**24 JAN 1953** Donor tissue:- Kill 3B5/1 M., born 1 Oct 52, and remove about  $\frac{1}{2}$  spleen,  $\frac{1}{4}$  of 1 kidney, and a testicle. Chop up fine with scissors; aspirate cells and tiny cell clumps into No.26 SWG needle on Agla syringe, spin down, and take up again to form a very dense suspension in Ringer. Inject 0.01 ml per foetus.

Recipients:- The nearterm foetuses representing the first litter of a female of litter 4A3/1, pregnant by litter mate. This mouse has been on supplementary diet (Milk, CLO, cheese, bacon rind, grape-nuts) since 17 Dec 52 and was segregated from its litter mates two days ago. Is nesting very well.

Open skin in midventral line by long incision and mobilize cautiously. Five (probably the whole lot) late foetuses were seen through body wall by gentle manipulations; the body wall itself was kept damp and not touched with instruments. These five foetuses were boldly injected intra-embryonically; injections of four were obviously successful, and probably also the fifth.

Skin prepared by shaving and germolene; no spirit. Multiple sutures to close skin, each touched with a dab of New-skin.

**25 JAN 1953**

Healthy litter of 3. No information about the other embryos. May be slightly premature.

<sup>FEB</sup>  
2 JAN 1953

2 healthy youngsters remain.

7 FEB 1953

Another young mouse has disappeared without a trace, and has probably been lost (dead or alive) whilst changing cage. The last survivor is very runtish and has a healed lesion in the genital region. (This could well be the site of inoculation.)

30 MAR 1953

Test operation.

Mouse 17 g. 8 weeks old.

Donor: Standard body-skin pinch graft from male of litter 4C2/2, b.6 Feb.

Graft bed prepared according to usual technique. Mouse with violet sacral identification mark.

7 APR 1953

(8) Note: animal has received cba test-graft, i.e. control skin. It will therefore have to be tested with aaUU skin in due course.

Healing of graft faultless. Graft slightly moist with pin-point haemorrhages. Gerolene. Rebandage.

10 APR 1953

(11) Epidermis peels away to reveal dermis with a yellowish-orange tinge. Scabbing takes place some time after removal of bandage. No survival.

2.

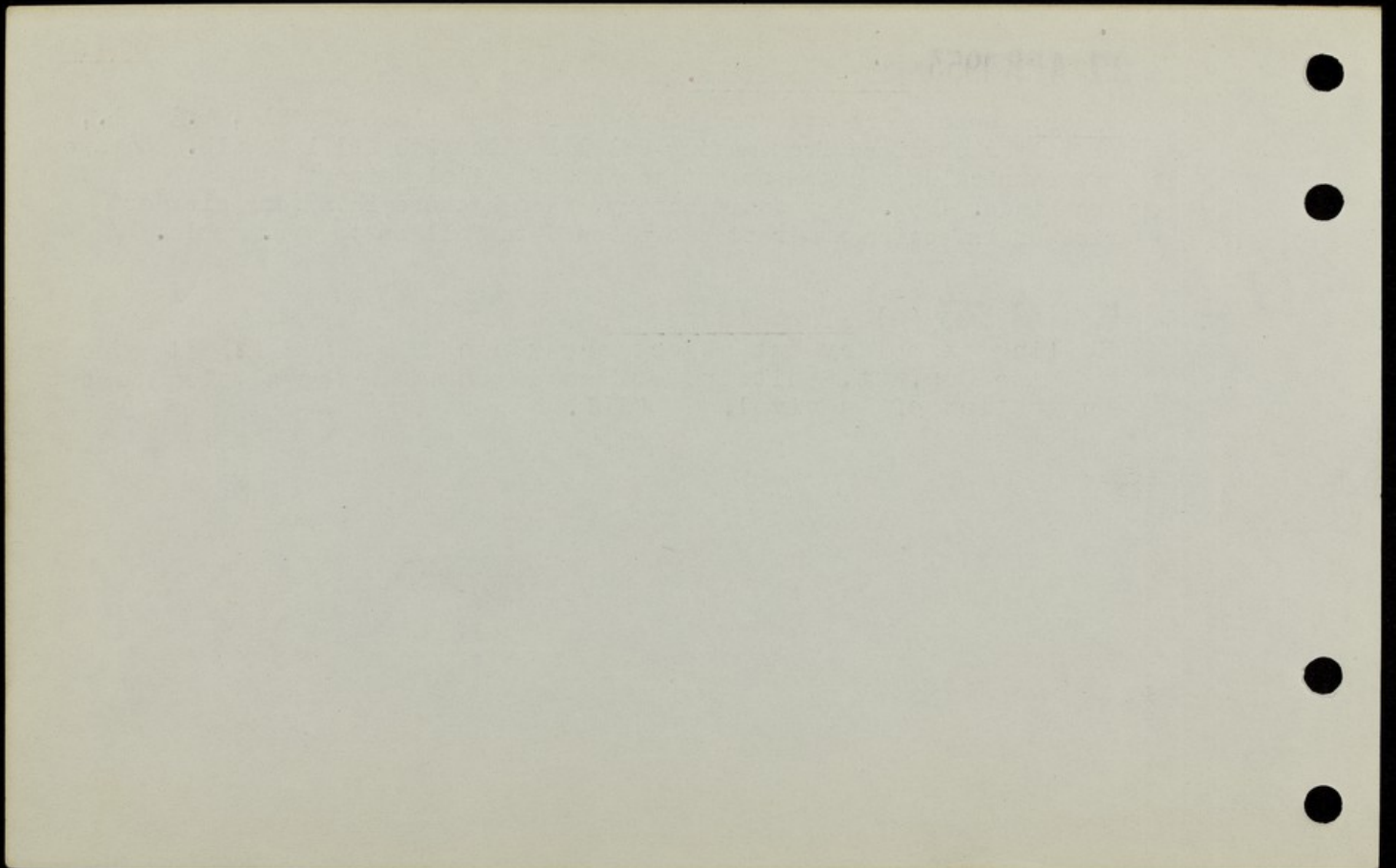
21 APR 1953 Test-operation.

EMB-69

Donor: Remove one standard pinch graft from flank of male, b.3 Feb., of litter obtained from mating of 3B3/1 male with 4B3/1 female. Scrape off panniculus and transplant to LHS of animal using the usual technique. Opn. very satisfactory, though there is a very slender risk of infection as graft was allowed to fall on to opn. table.

30 APR 1953 (9) First Inspection.

Healing exemplary but abject breakdown of the graft. It appears as a sodden, wet, white pad of collagen without the slightest suggestion of survival. KILL.





INJECTION OF FOETAL MICE (cba to A) INTRAEMBRYONICALLY WITH  
adult cell brei. INJECTIONS THROUGH PERITONEUM.

EMB-64

11 DEC 1952

Donor tissue: As for EMB-57 this day, except for the use of fine bore (26) needle. Very dense brei.

Recipient: Female of litter 3A2/4, o-parous, b. 27 Nov., 31 g. This mouse has been fed on a supplementar<sup>e</sup>v diet of cheese, milk, cod-liver oil and grape-nuts for the past fortnight. Segregated from litter 24 hours before operation.

NEW TECHNIQUE: The foetuses were injected through the mother's peritoneum which was allowed to remain intact. The body contents were manipulated by palpation of the peritoneum and as many foetuses as possible brought to the surface for intraperitoneal injection. The peritoneum was never touched with bare instruments and kept moist with Ringer.

5 embryos were thus injected, though 6-8 were probably present. Visualisation fair but not quite as good as in some previous ops. Needle seemed rather blunt and due to considerable difficulty experienced in piercing peritoneum, injections not too satisfactory. Some may have been intra-amniotic, and some cell-clumps found their way into the body-cavity of the mother.

Each injected foetus received 0.01 cc.

Nembutal anaesthesia without ether - very satisfactory. Mouse dried after ops. Germolene used, but no spirit in preparation of skin.

Good recovery after ops - animal seems fairly happy.

15 DEC 1952

HEALTHY LITTER BORN AT MIDDAY. Number unknown. Age of fetuses at time of injection therefore approx. 16 days. Mother nesting beautifully and seems to be looking after litter.

22 DEC 1952 The entire litter doing superlatively; being maintained on supplementary diet (including milk) the growth rate is unusually rapid.

30 JAN 1953

Test Operation.

Donor: Remove standard grafts from 4B3/1 male, b.6 Oct. Scrape off panniculus and store temporarily on Ringer-moistened filter-paper.

Recipients:

1. (R) M. 21 g. Ops O.k.
2. (L) M. 21 g. Ops O.K.
3. (no) M. 22 g. Ops O.K.
4. (both) M. 21 g. Ops O.K.
5. (2R) M. 22 g. Ops O.K.
6. (2L) M. 22 g. Ops O.K.
7. Female 17 g. Ops O.K.

Note: This litter was inadvertently test-operated with B-line skin. Hence wait until breakdown of grafts and use same bed for cba grafts. The present set of grafts will act as a control.

7 FEB 1953 (8)

EMB-64

2.

1. Healing faultless. Cuticle off with tulle. Roof dry, mat and undoubtedly epithelialised. Slight and rather superficial haemorrhage. Survival pretty considerable. Rebandage.
2. Healing faultless. Graft slightly puffy and rather more haemorrhagic than 1,3,5, and 6. But survival probably good.
3. Healing faultless. Cuticle off with tulle. Small scar dorsally and slight puffiness. Otherwise like 1 and 6 with good survival.
4. Healing faultless. Appearance of graft different from most of the others - scabbing dorsally, indications of extensive haemorrhage, with every sign of epithelial breakdown. Probably slight, if any, survival. Rebandage.
5. Healing faultless. Cuticle off with tulle. Exactly as for 1, 6 and 3. Rebandage.
6. Healing faultless. Cuticle off cleanly. On the whole like 1 but slightly puffy. Rebandage.
7. Healing faultless. Cuticle partly adherent. Roof haemorrhagic and moist - tending to patiness. Breakdown probably complete. Rebandage.

9 FEB 1953 (10)

All grafts obviously completely broken down. In most cases discoloration is extensive, and there can be no doubt that only the collagen pads remain.

Conclusion: If there should be a desensitised mouse in this litter this experiment will have shown clearly that desensitisation is

FEB 1953

a specific process. There may have been very slight prolongation since at the time of the 8 day inspection 5 out of the 7 grafts appeared to have considerable survival, whilst in the controls there is only trace-survival on the 8th day.

**12 FEB 1953** Test operation for C-line grafts.

Donor tissue: Standard pinch grafts (trimmed) from cba non-line female  
b.11 Nov.

1. M (R) 20 g. LHS Ops.O.K., but bed rather fatty.
2. M (L) 20 g. LHS "
3. M (no) 22 g. LHS "
4. M (both) 21 g. LHS "
5. M (2R) 22 g. RHS " Graft placed below B-line scab.
6. M (2L) 22 g. LHS "
7. F (no) 18 g. LHS "

**20 FEB 1953** (8)

1. Graft in position with moist surface, great erosion of dermis causing partial transparency. Impression after short exposure in air is that there might be some survival of weakly attached epidermis. Rebandage.
2. Very similar to 5. Faulty healing of dorsal 2/3 of graft where it is unattached and necrotic. Ventral 2/3 may be O.K. Rebandage.
3. Graft in place but posterior margin is free and there is some yellowish inspissated matter (↓). Greater part of graft attached - probably some survival of surface epithelium. Rebandaged.

4. Graft in place with margin united. Surface wet and pasty and dermis very thin in parts so that vessels of panniculus are showing. Looks like typical immune graft. Rebandage.
5. There seems to have been some type of technical failure. Ventral half of graft probably unattached to what appears to be a healthy and vascular bed - this portion of graft is damp whitish and obviously necrotic. Dorsal half seems to be reasonably healed in though it is somewhat inflamed. Powder and rebandage.
6. Graft is in place and reasonably well healed in. Surface moist and cuticle came away with reluctance in parts. On drying roof appears very weakly epithelialised but graft very inflamed.
7. Except for slight fault in healing ventrally graft looks perfectly O.K. Super-mouse? Rebandage.

Note: Healing has generally been extremely unsatisfactory, with the notable exception of 7. This may be due to

- a) infection
- b) technical failure
- c) immune effect
- d) a combination of the above.

As the litter was operated by L.B. and R.E.B. (that is by two operators) b) would appear to be rather unlikely. To check on a) a litter operated by the same individuals the previous day were inspected and they were found to have healed without fault. Whilst this does not, of course, rule out infection it does on the other hand make it very unlikely.

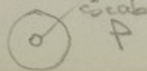
The general impression is that c), i.e. an immune effect, is responsible for the state of the grafts. This is a distinct possibility since these animals have already received B-line grafts. A cross-reaction is probable and will have to be investigated more fully.

If an immune effect is in operation than the state of the graft on 7 becomes all the more significant.

23 FEB 1953 (11)

1. Pick off slightly scabby cuticle to reveal dark pink graft area with central focus of ulceration. Area generally covered by thin and surviving epithelium which almost certainly consists of marginal ingrowth. Rebandage.
2. Pick off crusty material which probably represents remnant of graft. Area resurfaced by delicate epithelium which is is pretty certainly marginal autologous ingrowth. Complete contraction soon after exposure. Kill.
3. Mouse had removed bandage. Graft small scab. Kill.
4. Pull off scabby cuticle to reveal raw central area with some surviving epithelium vebtrally. This is probably ingrowth. Rebandage.
5. Pick off necrotic remains of graft which leaves raw central area surrounded by rim of ingrowing host epithelium. Kill.
6. Pick off dry scab from graft area. Much contraction. Slight bleeding from centre. Marginal ingrowth. Kill.
7. Definitely epithelial survival with central weakness and very small (and healing) scab. Whole graft epithelialised, but rather delicately. Rebandage.

A



A

26 FEB 1953 (14)

EMB-64

1. Graft area completely resurfaced by autologous epithelium. Contracture has taken place but not severely. Shortly after exposure severe contracture - no doubt that graft has gone. Kill.
2. Central point of ulceration, marginal ingrowth of host epithelium, severe contracture. Kill.
7. Graft somewhat contracted but weakly epithelialised. Central area of weakness particularly pronounced. But definitely fair survival over greater part of graft. Rebandage.

**2 MAR 1953** (18) Graft appears to be in position; it is epithelialised but somewhat puffy and generally showing signs of a mild reaction - unpigmented and not very robust epithelium, no follicular activity, a not very healthy deep pink colouration. BIOPSY.

Specimen EMB-64-7. (Note: the graft margins are not at all incisive and hence overgrowth ~~is~~ cannot be dismissed altogether though it is unlikely). Cut out slot with host skin at each end. Formol/Hg.

Histological report.

64.7. 100% survival. (18)

Graft extends over sections 1-4 only. There are several pigmented follicles (see section 1) which leave no doubt whatsoever that the epithelium belongs to the graft. There has been some contraction and hair-formation is not actively taking place. The dermis is

infiltrated by a light but uniform population of round cells which are completely viable - i.e. there is a light reaction. But no clear-cut symptoms of breakdown, and it is likely that this graft would have recovered in due course.

NOTE: Graft completely viable at 18 days, and this inspite of the fact that 14 days before it had been transplanted a B-line graft had broken down in normal way. This clearly points to the complete specifity of 'desensitisation'.

The other grafts in this litter either broke down or showed quite a strong immune effect. Experiments on the specificity of such an immune effect have shown very clearly that there is considerable overlap, and ~~indicates~~ that an animal which has born skin from one strain will show an immune effect when skin from a third strain is transplanted to it. Compare this finding with the now fairly firmly established fact of tolerance-specificity.



12 SEP 1952 DONOR TISSUE: Exactly as EMB-40, q.v.

RECIPIENTS: Foetuses of 0-parous cba female of litter 2C4/1, b.10 July, hole R ear post., pregnant by litter mate or first cousin.

6 + 2 foetuses **very near term** (18 days), each injected with 0.01 ml adult cell suspension subcutaneously. Visualization was exceedingly easy and there can be no doubt that the injections were successfully intra-embryonic. **OPEN ETHER ANAESTHESIA THROUGHOUT**. Animal well under, no ether during stitching procedures; but revival instantaneous, and mouse ran around & had a fall on recovery.

13 SEP 1952 Litter born 9.30 a.m.  
6 still-born. 2 alive. Mother suspected of failure to suckle properly, and the 2 young transferred to newly born litter of 4AI/P. (2 of the A-litter were given to the cba mother to suckle).

16 SEP 1952 One of the babies has died and disappeared. This was to be expected as it was in pretty bad shape when transferred to foster-mother. The other is doing fine, as are the 2 A-line babies fostered by cba ~~mother~~ mouse.

1 OCT 1952 The only survivor returned to its rightful mother., its foster litter-mates were considerably bigger and so had a decided advantage. The 2 A-line babies returned to their mother.

12 NOV 1952 TEST OPERATION.

DONOR: 2A4/2, male, b. II June. Preparation of grafts and beds as for EMB-38.

I. M. (no) 22 g. Fit fair.

20 NOV 1952 (8)

1. Graft is perfectly healed in, cuticle came off with tulle to reveal flat, supple, pink and epithelialized graft roof with only very slight indications of incipient haemorrhage. Rebandage.

24 NOV 1952 (12)

- I. Graft looks as perfect as any we have seen for many a moon. Surface well keratinised and healthy pink, and there is no trace of a reaction. Fine white hairs, possibly of new formation, can be distinguished with a lens. Rebandage, and inspect in 2 days.

26 NOV 1952 (14)

Graft healthy pink (perhaps a trifle too pink), uncontracted and quite obviously epithelialised and alive. Removal of flaky cuticle reveals clear-cut hair-growth, especially near the margins. Hairs white and rather downy, as yet very short.

After cleaning with spirit apply germoline to prevent scratching - no bandage.

27 NOV 1952 (15) GRAFT IN FINE FORM, and uncontracted. Short white hairs now easily visible. ~~0707~~ No sign of a reaction.

- 2 DEC 1952 (20) Graft healthy and growing a thick pelt of white hairs. Orientation of hairs is ventra-dorsal due to fortuitous rotation of graft on transplantation. No sign of a reaction. Photograph. (closed)
- 6 DEC 1952 (24) Graft O.K. Bushy hair-growth.
- 10 DEC 1952 (28) Mate with female of litter 3A4/3.
- 11 DEC 1952 (30) Graft O.K. Photograph mouse, then clip off graft hairs and surrounding fur and photograph again. Finally shave and photograph.
- 17 DEC 1952 (36) Graft O.K. - short white hairs beginning to appear. The graft itself has an exceedingly healthy appearance and is intimately linked with the surrounding host skin, but a distinct demarcation-line remains.  
Mate with female of litter 3C5/2, b. 11 Nov.
- 25 DEC 1952 (44) 3A4/3 female in advanced pregnancy; has been segregated 2 days now.

Condition of graft: A band of host skin as shown in fig 1 has been active for about a week; note that the wave of hair growth did not affect graft. Within past two days the



fig 1

rest of the field has become active, with growth of stout hairs; the graft is now sprouting dorsad hairs mainly from its dorsal half; these are pure white, but not yet of fully normal density. Fig 2.



f. 2

**30 DEC 1952** (28 + 20 = ~~48~~<sup>48</sup>). Further hair growth from graft. **Birth** of litter of 9 from the 3A4/3 female mated 10 Dec. Keep this for colour scoring; then destroy and remate female.

**6 JAN 1953** (55)

Litter from the 3A4/3 female uniformly agouti.

Graft: Has regrown a dense crop of long white hairs anteriorly and dorsally, with a few white hairs around the posterior rim. Rest of graft ventro-posteriorly uncontracted, healthy colour, clearly defined by ring of host hairs, and obviously epithelialised. Impression is that growth of graft hairs is gradually spreading over rest of graft.

**19 JAN 1953** (68) Secondary Test Operation: B-LINE GRAFT.

Donor: Male of litter 3B4/1, b.24 Sept.

Transplant standard graft to LHS thrax (dorso-lateral approach), using standard technique. Bed rather fatty - not all fat was removed. Operation O.K.

A-line graft: Condition quite unchanged. Outline of bald part of graft distinct - surface healthy. Rest of graft covered by dense, bushy hair-growth.

27 JAN 1953 (76)

EMB-41.

B-line graft: 8 day inspection.

Graft roof moist and haemorrhagic. Breakdown is advanced and survival, if any, is confined to a few small islets. Discolouration and scabbing on exposure. No need to re-bandage.

Conclusion: "Desensitisation" to A-line tissues is a strictly specific process and does not in any way influence the fate of homografts from another strain, in this case B-line.

A-line graft: Has lost much of its fur because the hairs adhered to the removed plaster. Trim hairs with scissors: graft outline clearly distinguishable, surface appears normal but graft may be very slightly swollen. Impression is that graft has contracted somewhat but not severely. These observations may well be due to ~~the~~ distortion resulting from the plaster bandage.

28 JAN 1953

B-line graft: Completely scabby.

A-line graft: (77) Some contraction has certainly taken place. There are one or two points of weakness but these are very probably due to local damage through removal of plaster. There are a number of white hairs and the roof looks pretty healthy on the whole.

The cba female is at last pregnant. Segregate.

30 JAN 1953 (79)

A-line graft: Graft is somewhat contracted to  $4\frac{1}{2}$  mm AP by 4 mm. Surface is perceptibly redder than host skin and is a little scaly. Nevertheless margin well defined, sparse white hairs still present and very firmly attached. Prognosis is continued survival, and present appearance may be due to chafing and irritation by plaster. No hair growth on field, hence not to be expected on graft.

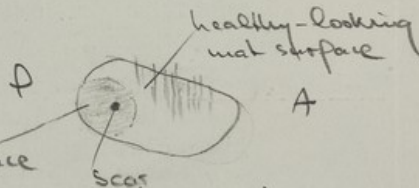
3 FEB 1953 (83) = 68 + 15

Remove A-line graft as a pinch graft (with panniculus and a little surrounding *gliding surface* host skin) and replace by standard pinch graft (trimmed) from female A-line mouse 3A3/1, b.9 July. Graft bed seems fairly satisfactory and ops. O.K. Good fit, graft rather larger than predecessor. Usual dressing.

Specimen EMB-41(A). Fix in Formol/Hg.

State of graft: Surface generally healthy-looking, but posteriorly there is a small scar surrounded by rather shiny epithelium. White hairs only dorsally, and even here very sparse. Impression is that the graft is viable but being subjected to an exceedingly mild reaction.

Weight of mouse 25 g.



4 FEB 1953

Litter from cba female of litter 3C5/2, b.11 Nov. EMB-41  
3 dead and mauled almost immediately, but 4 healthy survivors remain. These will be test-operated in due course.

11 FEB 1953

(8) Healing satisfactory. Slight annulus of granulation. Cuticle adherent - pull off cleanly. Graft brick red, and with signs of a violent inflammatory reaction. On the whole covered by a thin and unstable-looking epithelium, and there is a faint bleeding at one or two points. This appearance is very unlike both the 8 day controls and 2nd stage grafts.

13 FEB 1953

(10) Centre of graft ulcerated and graft as a whole very inflamed and moist. But possibly some marginal epithelial survival.

2nd litter from the 3A4/3 female is uniformly agouti and therefore destroyed.

17 FEB 1953

(14) Graft still showing very little indication of contraction. It is still quite patently in a bad way - very inflamed and red; BUT there are a few islets of epithelium which could represent

- a) surviving graft epithelium struggling against the reaction, or
- b) ingrowth by host epithelium.

The latter possibility is by no means unlikely and justifies

rebandaging and yet another inspection in 4 days time.

**21 FEB 1953** (18) Parts of the graft dermis remain in position but area resurfaced by ~~the~~ host epithelium. Contraction of area considerable. Graft has obviously disappeared. Leave unbandaged. Scabbing some time after exposure.

Time of breakdown impossible to assess., probably between 10 and 14 days.

**26 FEB 1953**

Tertiary test-operation.

It has been decided to transplant yet another A-line graft, this time on to a normally prepared bed on the LHS. An immune effect is to be expected.

Donor: 5A2/2, male, b.12 Jan. Remove one standard pinch graft, trim and transplant to scissor-prepared bed on RHS.

Inspect at 6 days.

**2 MAR 1953** The plaster bandage has unfortunately been gnawed just above the graft which is therefore scabby dorsally. The ventral half is relatively unaffected, though infection and faulty healing cannot be ruled out.

**5 MAR 1953** (7) Dorsal 1/3 scabby - cut this off. Below this area there is a raw patch, but some of it is epithelialised and it is



probable that the epithelium has been derived from the graft. EMB-41  
Rest of graft is soewhat darkish pink and is clearly surviving.  
Cuticle is adherent - leave.  
There does not appear to be an immune effect.

**7 MAR 1953** (9) Scabbing beginning anteriorly, must there is surviving epithelium over a fair area, especially posteriorly. The presence of some surviving epithelium rules out an immune effect. Rebandage.

**9 MAR 1953** (11) A scabby graft roof can be lifted off over the whole of the anterior and central portion, but posteriorly there seems to be a patch of surviving epidermis. Whether this is really so is almost impossible to ascertain - hence rebandage and re-examine in 2 days time.

**12 MAR 1953** (14) Cut off scabby roof noted at last inspection. Graft dermis is left in position, covered in patches by very thin epithelium. The latter is almost certainly due to overgrowth from the surrounding host skin. Apply germolene and leave unbandaged. **Keep animal** for yet another test-operation in 2 weeks time so that a clear-cut result can be obtained. Whilst demonstrating quite clearly that there has been no immune effect this graft has not been very satisfactory for the determination of survival - damage due to gnawing (nearly half of original graft was lost) and possible subsequent infection complicated the whole picture too much.

Animal certainly fertile - female of litter 3C5/2 again pregnant.

18 MAR 1953 Litter from 305/2. 6 young.

31 MAR 1953 Last and final test-operation!

Donor: 4A4/3, male, b.12 Feb. Remove standard body-skin graft in usual way, trim. Prepare bed on RHS using scissors method. Mouse 28 g.

3 APR 1953 Destroy 305/2 female, which is again pregnant.  
The complete fertility of EMB-41 has been adequately proved.

7 APR 1953 (7) Well healed but ghost adherent. Graft swollen and flushed, with distinct haemorrhages. Rim secure. Definitely some surviving epithelium - hence **NO IMMUNE EFFECT**. Rebandage.

10 APR 1953 (10) Graft has rather dark red colour, especially dorsally and ventrally. Ghost and hairs surprisingly adherent, but surviving epithelium appears to be present over entire graft. Germolene only.

11 APR 1953 (11) Most of ~~the~~ graft is card-boardly though not grossly discoloured. Probably still some survival. No typical scabbing effect.

14 APR 1953 (14) At no time has typical scabbing been observed. The graft has contracted somewhat, looks rather leathery, but it is not inconceivable that some surviving epithelium remains. To check this possibility sacrifice animal.

Specimen EMB-41(2). Cut out slot with host skin at each end. Formol/Hg.

Histological report.

EMB-41

41.A. 90% survival. (83)

Breakdown has occurred over a small area near middle of graft, but it is very local and even here there is a high degree of follicular survival. In this region round cell infiltration is considerable, whilst elsewhere it is very light to moderate. Vascularisation fair. Graft epithelium is healthy though some cells are slightly vacuolated. Note many new follicles and sebaceous gland tissue, no sign of pigmentation (this is in marked contrast ~~with~~ to the pigmented host follicles).

For photography, note group of follicles on penultimate section.

Conclusion: this is a viable graft with a very mild reaction.

41.2. 100% survival. (14)

Hyperplasia of epidermis, cystic dilatation of follicles, heavy round cell infiltration into the graft bed and to a lesser extent into the deeper dermis, but invading cells are still viable. Erosion of some deep follicular tissue and some superficial epidermis is about to begin.

Conclusion: Graft completely viable, but reaction building up.

NOTE: 1st graft biopsied on 83 day - almost complete survival.  
2nd graft transplanted on same day - 8 days appearance very abnormal, but not immune. Survival-time probably 10-14 days.  
3rd graft transplanted on 83 + 23. Graft gnawed and result therefore inconclusive, but no immune effect.

4th graft transplanted on 83 + 23 + 33. At 14 days biopsy this graft shows a 100% survival.

Hence animals tolerance to A-line tissue still existed 6 months after the 'desensitising' injection, and this inspite of the fact that during this time at least one graft broke down. No immune effect has been observed at any stage.

Furthermore, the score of 41.A shows clearly that the 'tolerance' is highly specific in nature: 7 days before its biopsy a B-line graft had broken down in a normal way.

INOCULATION OF FOETAL MICE (CBA to A) WITH ADULT WHOLE BLOOD

EMB-180

8 MAR 1954 Donor: As for EMB-176.

Recipients: Foetuses of female 8A5/P. Multi-parous. No mark.

7 foetuses very near term. 0.01 cc. Injections very reliably intraembryonic.

9 MAR 1954 Healthy litter of 6 born overnight - about 12 hrs after injection. 2 of these are visibly injected mice.

26 APR 1954 Test-operation.

Donor: CBA stock 14 female. Recipients: 6 mice.

7 MAY 1954 (11) 4/6 with completed breakdown. Kill.  
2/6 with 100% survival and light pigmentation.

9 MAY 1954 (13) R. B.d. nearly complete - slight central survival.  
L. 100% survival, but still delicate.  
Light pigmentation.

11 MAY 1954 (15) R. B.d. complete. Score: 14 days.  
L. 100%. Incipient hairgrowth.

15 MAY 1954 (19) Graft on L notch is O.K. and growing agouti hairs, but note that graft went through early contracture, and that hairgrowth is abnormally slow.

27 MAY 1954 (31) Marginal scabbing in progress. Graft clearly on way out.

31 MAY 1954 (34) Breakdown complete. Score: 33 days.

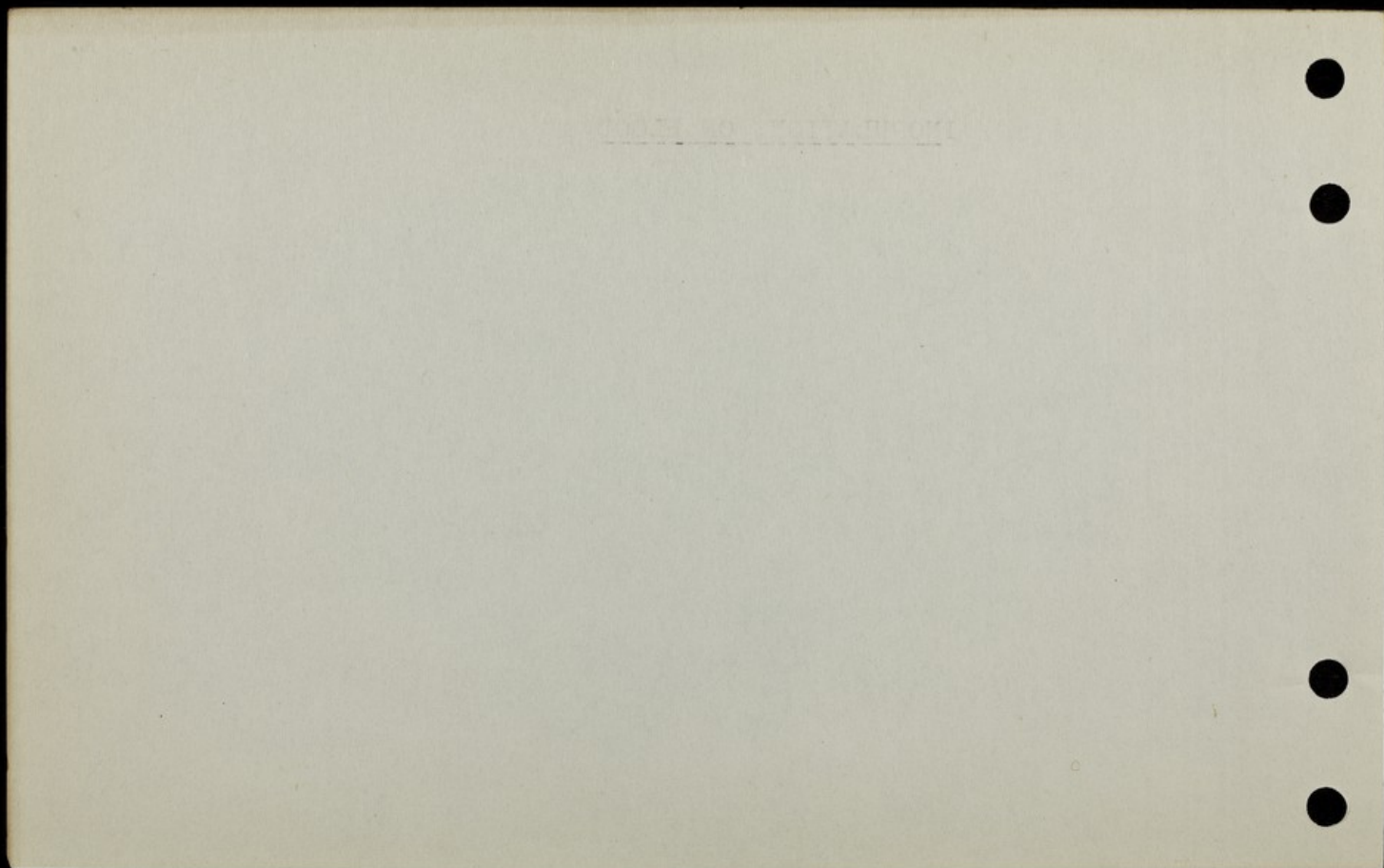
1 JUN 1954 (35) SECOND STAGE OPERATION. Donor: CBA XVI male. LHS.

10 JUN 1954 (35 + 9) Graftwell healed; rim fully attached. Surface yellowy-brown after clean complete separation of 'ghost' (indicating hyperplasia at earlier stage). Just a hint of trace survival of epithelium: at all events, a breakdown time of 9 days can be accepted. (Normal expectation less than 6 days).

EXPERIMENT CONCLUDED

B.

INOCULATIONS OF BLOOD





2 JUN 1954 INOCULUM:- As for EMB-216.

RECIPIENT:- 10A6/1. 31 g. See clearly and quite certainly inject 4 x 18-day foetuses, each with 0.02 ml inoculum. Too late for good result.

3 JUN 1954 ~~Four~~<sup>Six</sup> born overnight 2/3 June (say 12 hrs post-op.).

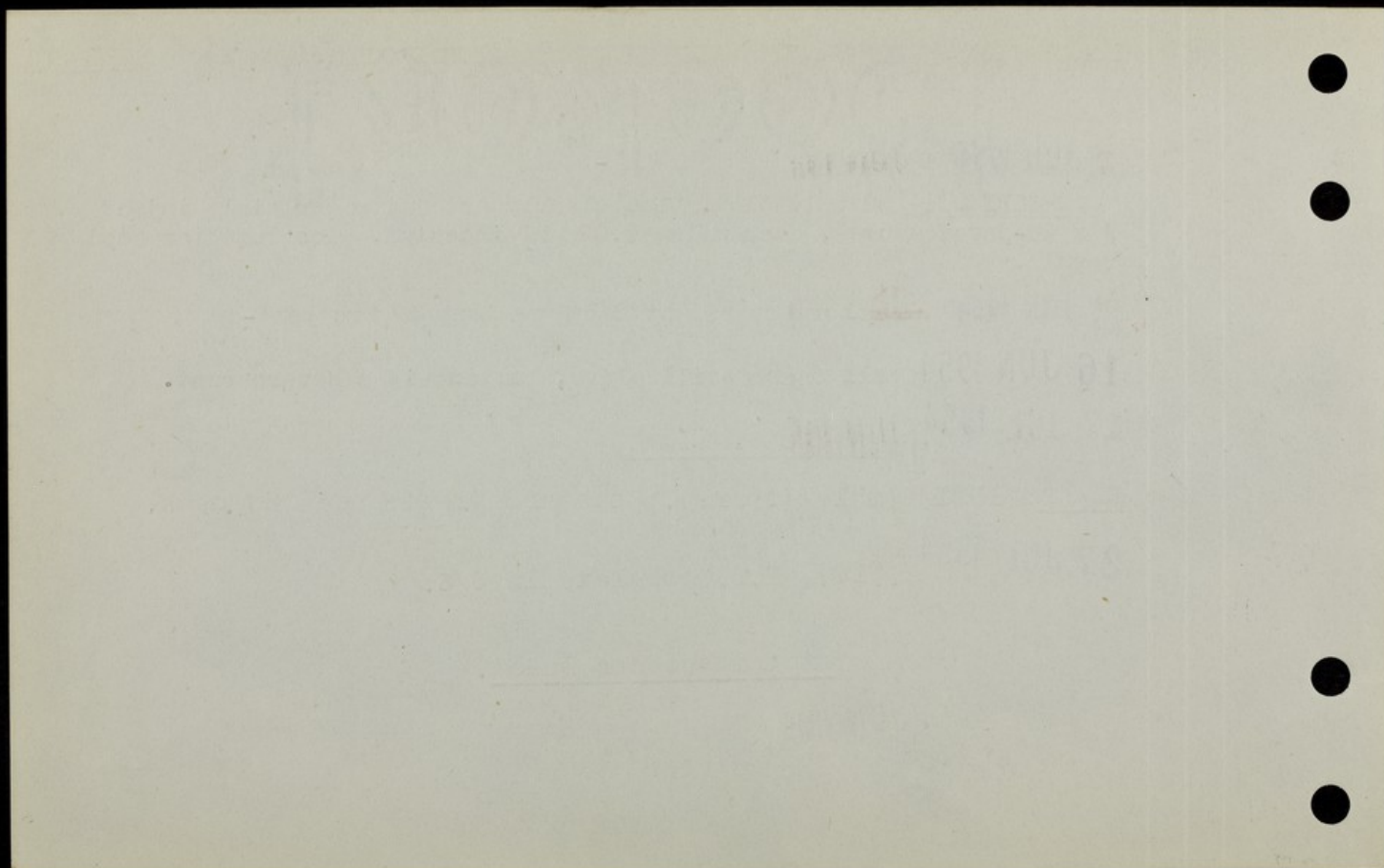
16 JUN 1954 All 6 are still alive, but one is a severe runt.

17 JUL 1954 Test-operation.

Donor: 10B1/2 male (spare). Standard grafts and RHS opns.

27 JUL 1954 (10) B.D. complete in 6/6.

Experiment concluded.



17 MAY 1954 INOCULUM: As EMB-214.

RECIPIENT:- Foetuses representing 2nd litter of 10A2/1 F., 5 embryos, 17 days, each getting 0.01 ml, injections pretty reliable.

22 MAY 1954 Litter born overnight - 2 alive and 3 dead.  
Age at injection therefore about 16 days.

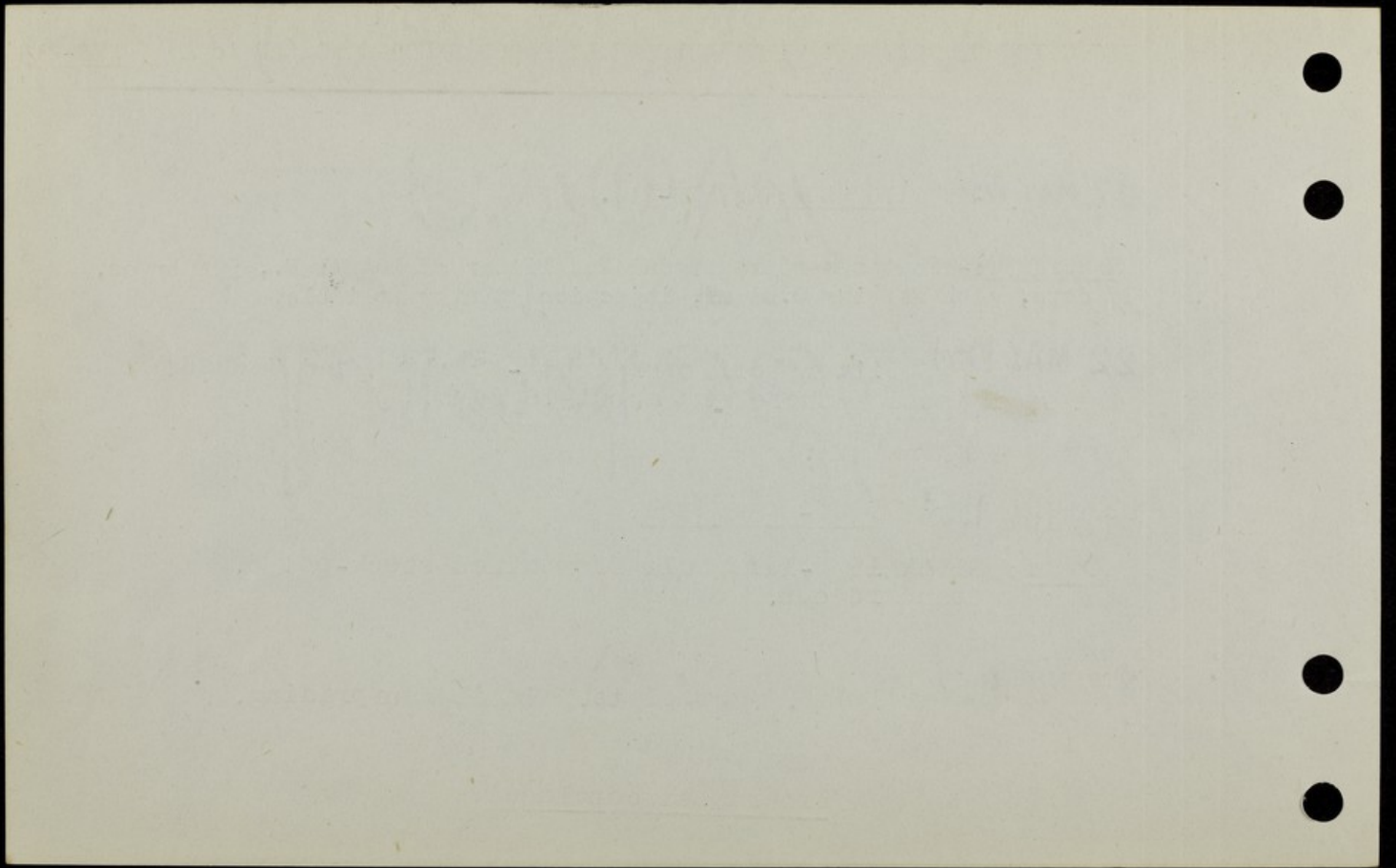
30 MAY 1954 ~~One~~ left.

13 JUL 1954 Test-operation.

Donor: ~~Stock 12~~ B-line male from mixed stock-pot 6.  
Standard opn.

23 JUL 1954 (10) B.D. complete. Hardly surprising.

Experiment concluded.



INJECTION OF FOETAL MICE WITH LEUCOCYTE-RICH WHOLE BLOOD (AU to A) EMB-204

**8 MAY 1954 INOCULUM:-** Take 1.8 ml whole blood from two AU-mice (either 8B4/1 or 7B5/3), and mix in narrow centrifuge tube with 0.2 ml 4% citrate. Spin down flat; take off buffy coat with top layer of red cells, all suspended in autologous citrated plasma. Spinning unduly prolonged, hence some platelet clumping, but leucocytes almost entirely unagglutinated.

|                                                    |                                 |
|----------------------------------------------------|---------------------------------|
| <b>COUNT;-</b> red cells 2,700,000/mm <sup>3</sup> | I.e. a two-three time conc. of  |
| whites 18,400/mm <sup>3</sup>                      | leucocytes with half normal no. |
|                                                    | of red cells.                   |

**RECIPRO-**parous F of litter 8A5/4. 35 g. See clearly and inject 7 foetuses, 18+ days, 0.01 each (some 'a little more'). Good operation.

**10 MAY 1954** Litter of seven (complete yield) born overnight 9/10 May, i.e. 36 hr postoperatively. One still shows free blood in peritoneal cavity, & probably also a second.

**30 MAY 1954** Six still alive, though somewhat backward and not being well looked after.

**16 JUN 1954** 6 still alive. 6

13 JUL 1954

Test-operation.

Donor: B-line male from mixed stock-pot 6. Standard opn. on RHS  
of all 6 recipis.

23 JUL 1954

(10) All 6 grafts: b.d. complete.

This is very surprisng, and speaks strongly a  
against the efficacy of leucocyte-rich blood.

Experiment concluded.

INJECTION OF FOETAL MICE WITH ADULT CELLS (AU to A)  
(LEUCOCYTE-RICH)

EMB-203

3 MAY 1954 Inoculum:- As for EMB-200.

Recipient:- 10A4/2 litter, 31 g. Inject accurately six foetuses at term with 0.01 ml.

4 MAY 1954 Litter of 6 born overnight, i.e. about 12 hr after injection. May be slightly premature, but foetuses look all right and were being cared for.

10 MAY 1954 The six doing well.

30 MAY 1954 One left and very feeble. 2

13 JUL 1954 Test-operation.

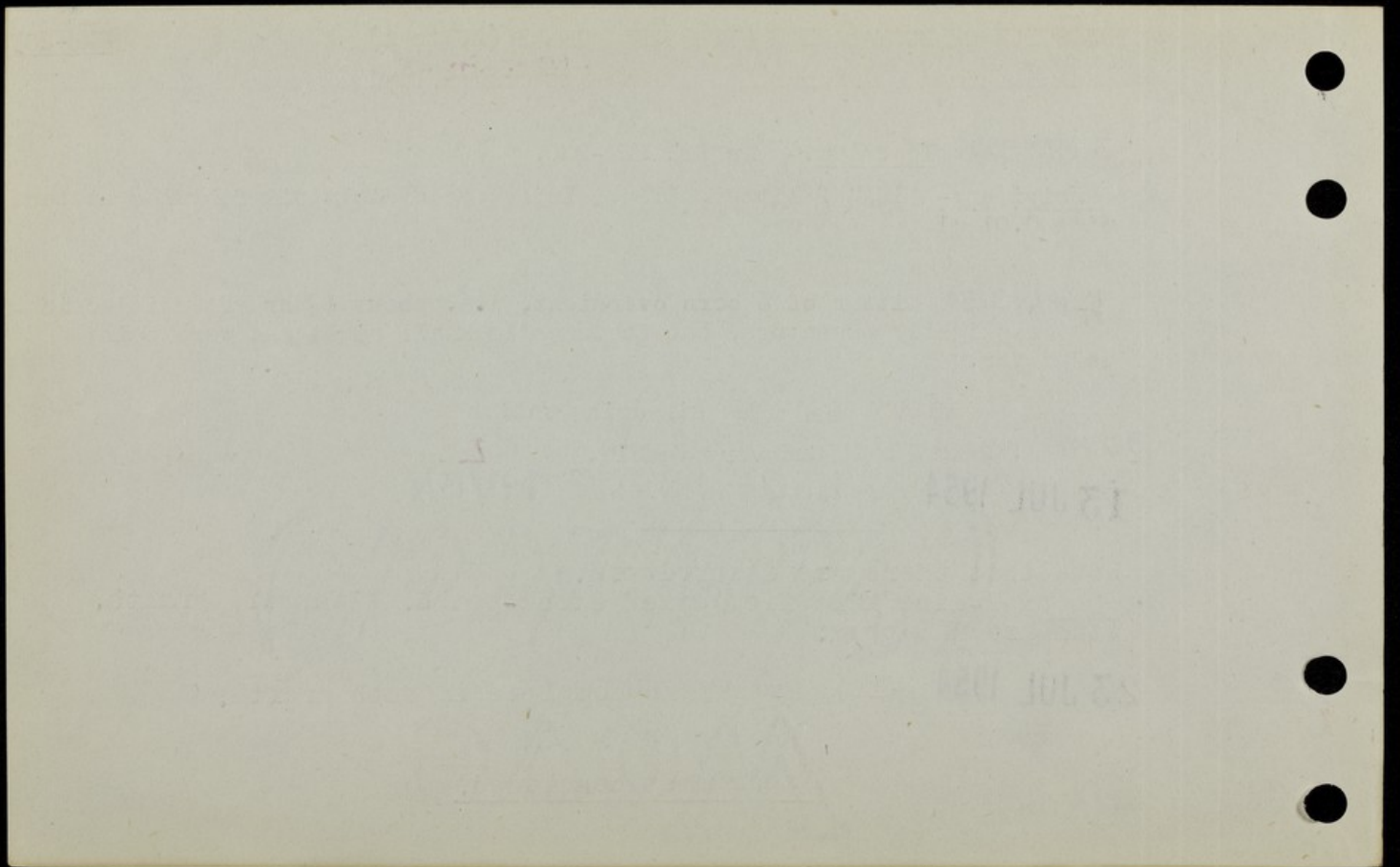
Note that there are 2 survivors.

Donor: B-line male from mixed stock-pot 6. Standard grafts, standard RHS opns.

23 JUL 1954 (10) Breakdown complete in both grafts.

Experiment concluded.

UL





INJECTION OF FOETAL MICE WITH ADULT BLOOD(AU to A)  
LEUCOCYTE-RICH

EBM-201

3 MAY 1954 Inoculum: As for EMB-200.

Recipient:- 8A7/2 litter, 32 g. Foetuses 15-16 days. 4 well injected, 2 blind. 0.005 ml each. Postoperatively, mouse restless and not nesting well.

8 May 1954 Litter of eight born overnight 7/8, 3 live, 3 dead (of which one long dead), 2 dying (and later died). Thus birth  $4\frac{1}{2}$  days post op.

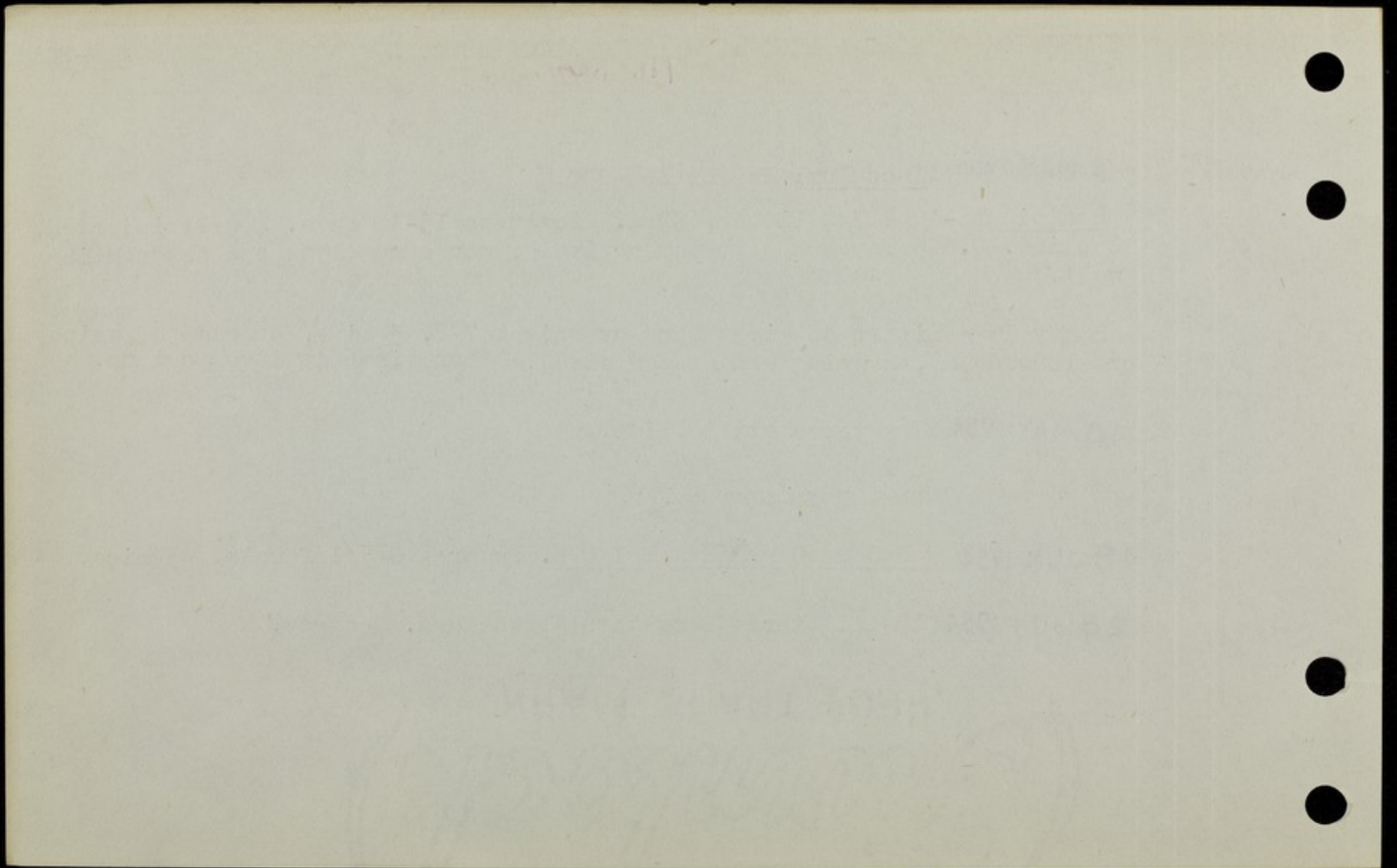
10 MAY 1954 The three still alive.

30 MAY 1954 Three survivors still.

18 JUN 1954 TEST OPERATION. Donor:- 8B5/1 female. 3 mice.

28 JUN 1954 (10) All three show total breakdown. Destroy.

EXPERIMENT CONCLUDED



INOCULATIONS OF FOETAL MICE (A to CBA) WITH ADULT WHOLE BLOOD EMB-179

**8 MAR 1954** Donor: Citrated whole blood from A-line Stock 11 male.  
Recipients: Foetuses from female 8C3/2, b.10 Nov. 1-parous.  
3 + 3 -- about 18 days. 0.01 cc. Injections very good.

**10 MAR 1954** Healthy litter of 6 born overnight - age at time of injection about 17-18 days.

~~12 MAR 1954~~

**26 APR 1954** Test-operation.

Donor: A-line ~~8A5/4~~ mouse from 8A5/4. Recipients: 6 mice.

**7 MAY 1954** (11) 5/6 with completed breakdown. Kill.  
1/6 with 100% survival, but a little delicate.

**9 MAY 1954** (13) Graft with 100% survival. Graft fairly robust-looking.

**11 MAY 1954** (15) 100%, but very small marginal scabs have

appeared, and the graft is somewhat swollen. Bald.

**15 MAY 1954** (19) Graft undergoing ~~the~~ slow scabbing. Breakdown now far advanced.

(11)

INOCULATION OF FOETUSES (CBA to A) WITH ADULT WHOLE BLOOD

EMB-177

**8 MAR 1954** Donor: As for EMB-176.

Recipients: Foetuses of female from litter 8A1/4. b.2 Dec. 1-parous. Rea  
Visualisation good but details of operation have been lost.  
It is likely that age was fairly advanced; number uncertain, but not  
less than 5.

**10 MAR 1954** Litter born early this afternoon - 5 alive, 1 dead, and  
1 clearly destined to die with cyanosed appearance and gasping.  
Age at time of injection 17-18 days.

**26 APR 1954** Test-operation.

Donor: Stock 14 CBA females. Recipients: 5 mice.

**27 MAY 1954** (11) 2/5 with complete breakdown.  
3/5 with 100% survival: L,R, no. Gent.viol.  
base of tail.

**29 MAY 1954** (13) L. B.d. far advanced - slight survival.  
R. High degree of survival, but marginal scabbing.  
No. Extensive marginal scabbing. 50% survival.

11 MAY 1954 (15) L. & No. B.d. complete. Kill.  
Scores; 14 days.

R. About 50% survival. Light marginal scabbing. Graft  
lightly pigmented. A few agouti hairs have penetrated.

14 MAY 1954 (18) B.d. just complete.  
Score: 18 days. kill.

**6 FEB 1954** DONOR:- AS EMB-165.

RECIP:- F of breeding pair<sup>8A2/P</sup> in 7th pregnancy. (Last litter 8A2/6.1-6 = EMB-154.) 0.01 ml in each of 3+3 15/16 day fetuses. Presentation poor & only one injected with complete certainty. R ear, 45 g.

**10 FEB 1954** Healthy litter of 5 born overnight, i.e. 3½ days after injection. Encouraging, but note operational note.

**26 MAR 1954** TEST OPERATION. Donor: 8B1/3 male.

**15 APR 1954** (10) 1/5 with complete breakdown. Kill.  
4/5 with 100% survival. Number as follows:

**Both ears and L:** grafts rather puffy and scurfy and pink, but robustly endothelialised.

**R:** Just perceptible pigmentation. Also very slight vascular dilatation.

**No:** Best graft, completely uncontracted and with heavy pigmentary shadow.

**6 APR 1954** (11) **R.** 100% survival. Pigmentation.

**L.** Very high degree of survival but slight scabbing ventrally.

**No.** 100% - some pigmentation, but very faint spotty discolouration.

Both. High degree of survival but some scabbing and contraction.

- 7 APR 1954 (12) Little change in any of these grafts.
- 8 APR 1954 (13) 2/3 survivors are in perfect condition: flat, supple, uncontracted, firmly epithelialised, and patches of intense pigmentation.  
1/3 has great deal of survival, but central scab which indicates reaction - but marginal pigmented survival.
- 9 APR 1954 (14) 2/3 perfect. One has small tuft of erupting hairs.  
1/3 with b.d. slowly continuing, but still marginal pigmented survival.
- 14 APR 1954 (19) 1/3 with breakdown complete. Score: 18 days  
2/3 are in perfect condition. Both grafts are completely uncontracted and robustly epithelialised, with several patches of intense pigmentation which have produced dense hair-crop. Pigmentation is now spreading over the whole of the previously pale grafts.
- 17 APR 1954 (22) Both grafts with bushy black pelts of hair.  
Note that one of these mice suffers from middle ear trouble, and cannot balance properly.



EMB-167

26 APR 1954 (31)

The seedy mouse, now rather emaciated, has died. Store in frig. overnight, and PHOTOGRAPH. (Call mouse EMB-167.1.) Then remove graft for histology.

Specimen, EMB-167.1.

(PHOTOGRAPH BLACK & WHITE)

No.2 is doing well.

Both grafts are in excellent shape and without the slightest sign of a reaction. Both have thick pelt of hairs (black).

3 MAY 1954 (38) No.2 is suspiciously docile and subdued; fur rather staring, and appearance hunched. Very little likelihood of survival.

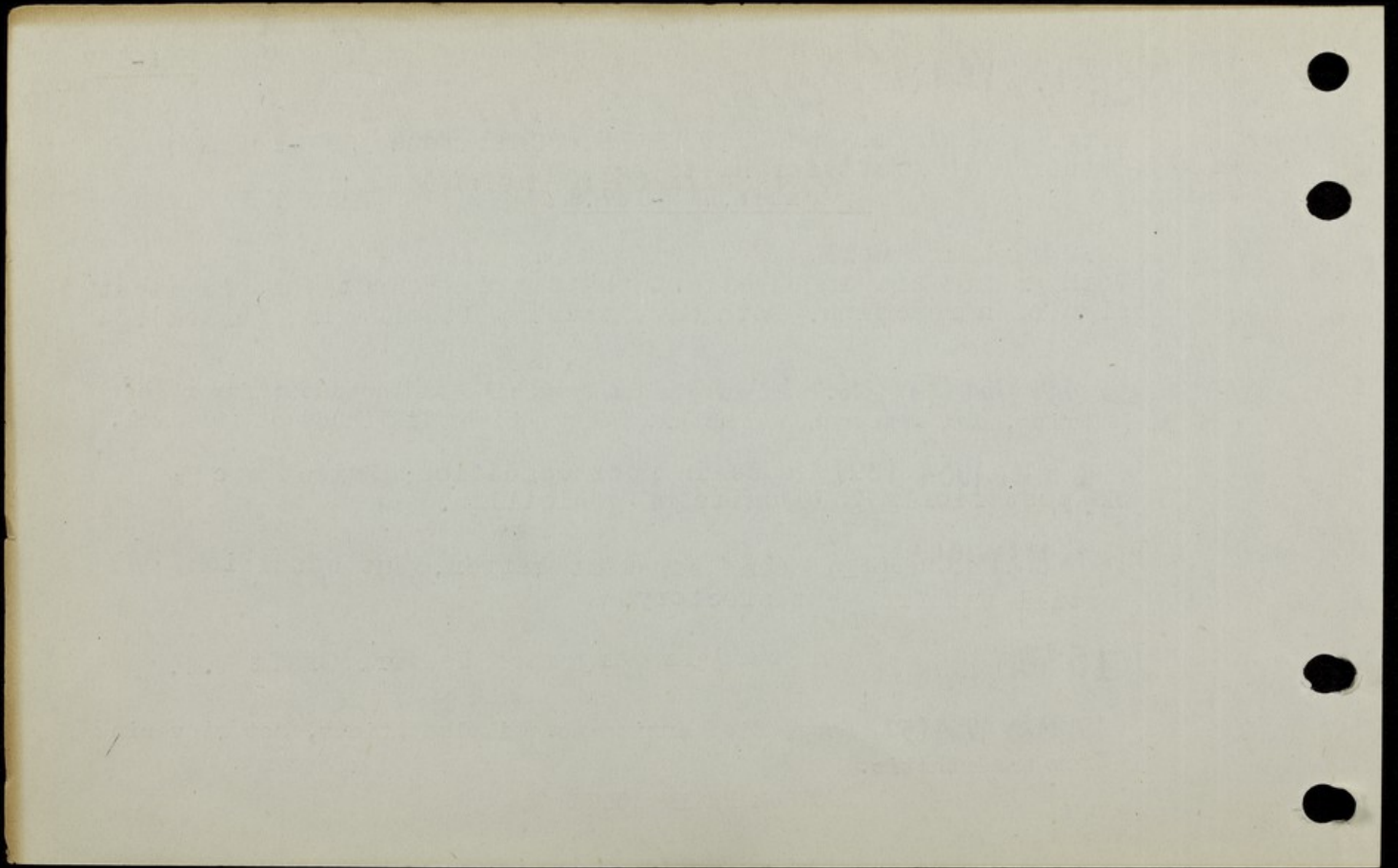
4 MAY 1954 (39) Mouse in poor condition: Inject s/c LHS posteriorly 7500 units of penicillin.

6 MAY 1954 (41) Mouse somewhat better, but condition still far from satisfactory.

15 MAY 1954 (50) Mouse is very much better. Graft O.K.

17 MAY 1954 (52) Mouse died during node implantations, not reovering from anaesthetic.

PHOTOGRAPH IN COLOUR



**6 FEB 1954** DONOR: - As EMB-165.

RECIP: - F of breeding pair 8A1/P, in 5th pregnancy. 52g, L ear. 0.01 ml into each of 6 18-day foetuses (one inj. uncertain). Possibly 7th foetus dead in membranes. [See EMB-191]

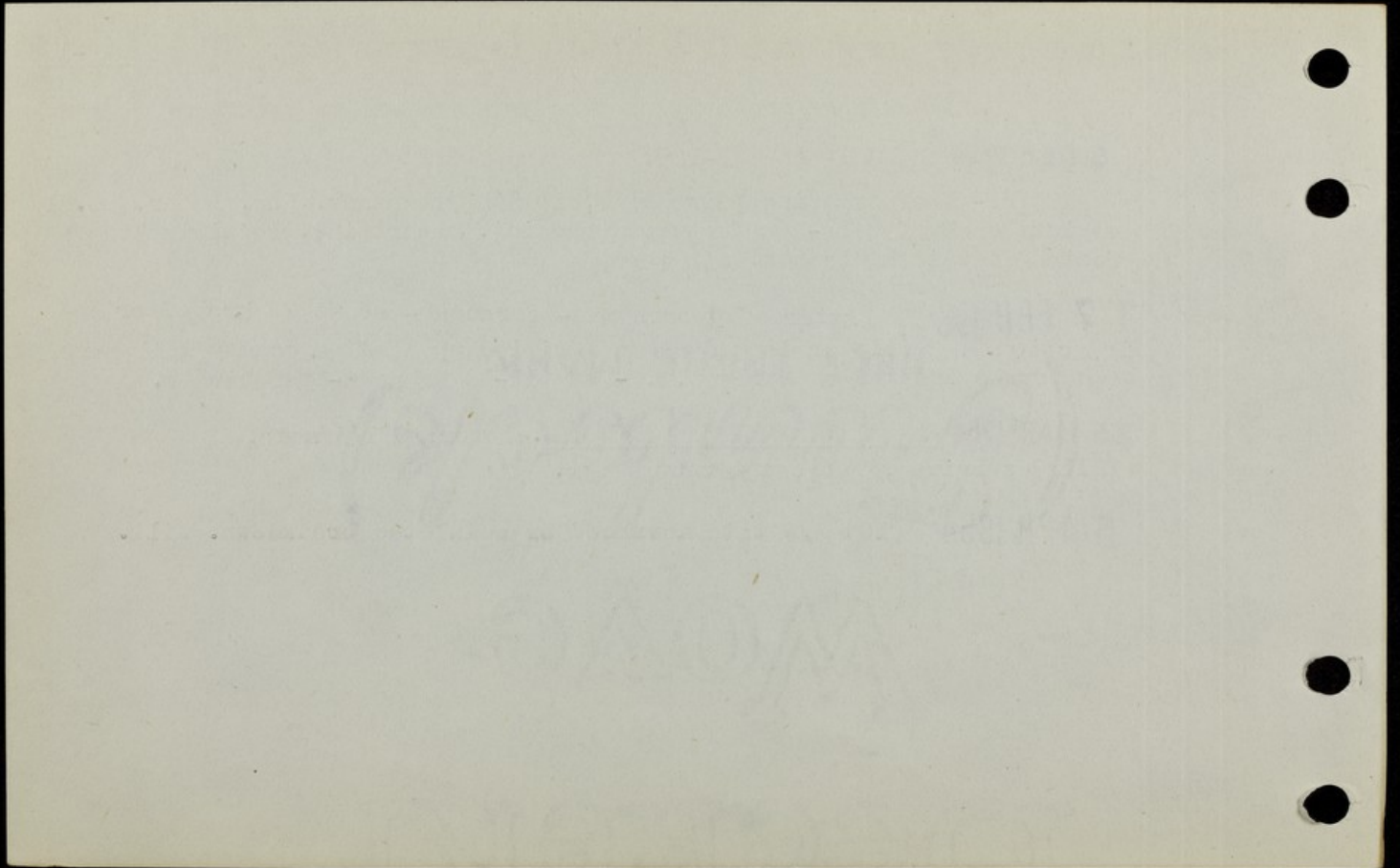
**7 FEB 1954** Litter of 9 born mid-afternoon - about 24 hrs after injection. (Litter looked clean & tidied up at 4 pm so may well have been born overnight or early a.m. - say 12-18 hr post-operatively.)

**26 MAR 1954** TEST OPERATION. Donor: 8B1/3 male.

[All 9 survived, but one a runt: 11 g.]

**5 APR 1954** (10) 9/9 with advanced or completed breakdown. Kill.

**6 APR 1954** (11)



**3 DEC 1953** DONOR:- Exactly as for EMB-145.

RECIPIENT:- Exactly as for EMB-145. 34 g., no notch.

Seven foetuses seen and injected with 0.02 ml each. Uterine horns very mobile & hence visualization pretty good for this ~~age~~ age (15-16 days). Injections satisfactory.

**7 DEC 1953** Litter of 3 found to-day, but they were most probably born yesterday. Age at time of injection therefore about 16 days.  
No trace of rest of litter - likelihood that they were resorbed as mother became 'less pregnant' after operation.

**20 JAN 1954** Test-operation.

Donor: Stock 13 cba male. Standard grafts.

Operate all 3 survivors - RHS.

**31 JAN 1954** (11) Primary inspection.

2/3 grafts show total breakdown.

1/3 has 100% survival and is lightly pigmented - **Super-mouse?**

**1 FEB 1954** The survivor (L ear notch) is fully epithelialised and still pigmented - most promising. Keep together with EMB-145.

5 FEB 1954 (16) Graft still fully surviving, pigmented, and just beginning to grow cba hairs. Hair-growth is a little late and feeble, but at last it is possible to be reasonably certain that this is a fully tolerant mouse.

6 FEB 1954 (17) No further progress: some contracture?

18 FEB 1954 (19) Breakdown is almost complete - severe scabbing has taken place and there remains little surviving epithelium.

Score: 19 days.

**3 DEC 1953** DONOR:- Withdraw 0.8 whole blood from heart into citrate damped syringe and mix at once with about 0.08 ml 4% citrate. Donor:- male of 5C5/P breeding pair.

RECIPIENT:- Foetuses of Stock V A-line female, in second pregnancy by 7A1/5 male, S.D., 39g, R ear notch.

8 foetuses seen; 7 injected, 6 of which really satisfactorily. 0.04 ml each. Age about 17 days. Visualization good.

**5 DEC 1953** Healthy litter of 7-8 born overnight - mother nervous so do not disturb to look for injected blood. Age at time of injection about 17 days. This ought to be a promising experiment.

**7 DEC 1953** Only 4 survivors remain - no trace of the rest. One is unlikely to survive as it is an extreme runt.

*7 young will. ✓*

**20 JAN 1954** Test-operation.

Donor: Stock ~~8&&&&~~ 13 cba male. Standard grafts.

Operate all 7 mice. RHS opns. Weights 18-20 g.

**31 JAN 1954** (11) Inspection.

5/7 grafts show total b.d. or merely trace-survival.

2/7 have 100% survival of the epithelium, but of these one has patches with epidermal weakness.

**1 FEB 1954** (12) 1 graft fully surviving, and deeply pigmented. **Super-mouse?**

2nd graft predominantly surviving, but slight superficial scabbing. There is just a perceptible shadow of pigmentation in centre.

**4 FEB 1954** (15) One graft has now broken down - **score 14 days.**

The remaining survivor is growing a thick pelt of hairs, its skin being particularly active. Clearly a **fully tolerant mouse.**

**6 FEB 1954** (16) No further progress; graft somewhat hard, with rim scab; definition indication of slight hold-up or regression.

**8 FEB 1954** (18) Graft is undoubtedly going through a powerful reaction: There has been considerable contracture and marginal scabbing. Graft palpable.

**10 FEB 1954** (20) Breakdown now complete - destroy.

**Scores: 14 and 20 days.**



20 NOV 1953

Donor: A-line Stock 7 male. By cardiac puncture withdraw 0.4 cc whole blood into syringe sparsely wetted with 4% citrate $\frac{1}{2}$  and immediately mix with 0.04 cc 4% citrate and stir. Inject through tuberculin syringe with very fine needle, the syringe itself having been slightly wetted with citrate before loading with blood.

Recipients: Cba Stock 2 female, long-term special diet, pregnant by litter-mate, 39 g., 2nd pregnancy.

Foetuses very near term. 6 seen and injected intra-embryonically with 0.05 cc of whole blood. All injections were successful, some superficial.

Apart from age of embryos operation not unhopeful.

21 NOV 1953

Healthy litter of 8 born overnight. 5 of these are clearly marked by large haemotoma in various parts of the body and have obviously been successfully injected. A 6th appears to have some discoloration around the region of the liver and may well have been injected. Since the litter is rather large, destroy the 2 mice which have escaped injection.

23 NOV 1953 Litter doing well.

~~8~~ JAN 1954 Test-operation.

Donor: A-line Stock 7 male. Standard grafts. Operate RHS of all 6 mice.

18 JAN 1954 (10) - Inspection. 4/6 total breakdown complicated by a severe macerating infection.

1/6 shows infected corner V/P; rest shows high degree of survival, but breakdown beginning (epidermis peels centrally).

1/6 100% survival at present: matt appearance. ?Supermouse.

21 JAN 1954 (13) 1 graft has almost completely scabbed but still has a dorsal patch of survival.

The other graft has scabbed dorsal half, but its ventral half is fully surviving.

23 JAN 1954 (15) One graft with b.d. just about complete. KILL.  
The other graft still has a small patch of survival.

25 JAN 1954 (17) Still small patch of ventral survival.

29 JAN 1954 (21) Survival is possible in a small patch, but it is more likely that this is scar tissue.

Scores in this expmt. as follows: 15 days

NOTE WHOLE BLOOD.

20 days

**13 NOV 1953** Donor blood: By cardiac puncture of 3 Stock VIII CBA females, withdraw 1.3 ml whole blood into syringe sparsely wetted with 4% citrate, and at once mix with 0.15 ml 4% citrate & stir. Inject through tuberculin syringe with finest possible needle, the syringe itself having been wetted before the first filling only with 4% citrate. No clotting, and barring possible toxicity of citrate, technique should be O.K. See also EMB-138-9.

Recipient: 39g Stock V A-line female pregnant by Stock V A-line male (possibly Stock IV male), in second pregnancy, notch right ear.

Usual exposure (skin shaved & germolened). Visualization very good; foetuses 17-18 days old; 8 injections, five of which were certainly OK. Each received 0.05 ml. Possibly one foetus injected twice.

The injections were generally intraembryonic rather than subcutaneous.

**15 NOV 1953** Litter of 4 first seen late this a.m. **Two** of these have gross and obvious discoloration due to injected blood.

**23 NOV 1953** Litter doing well.

**8 JAN** Test-operation.

Donor: Stock 7 male cba - standard grafts. Operate RHS of all 4 mice.

18 JAN 1954(10) - Inspection. 3/4 total breakdown. 1/4 (female in advanced pregnancy): graft with completely surviving epithelium, rather white (usual pregnancy effect). Streak of reddening D/V. ?Supermouse.

21 JAN 1954 (13) Graft predominantly surviving and uncontracted, but there is a large anterior and a small posterior marginal scab.

23 JAN 1954 (15) Graft surviving and fairly uncontracted, but anterior scab still present. Rather white.

25 JAN 1954 (17) Posterior half of graft still fully surviving, but no hair-growth; progressive scabbing appears to be in progress.

29 JAN 1954 (21) There is still a small patch of clear-cut survival, but graft has scabbed progressively and b.d. cannot be far off.

30 ~~FEB~~ JAN 1954 (22) For safety's sake this graft must be scored as b.d. from to-day.  
Score: 22 days.

**7 JUL 1954** DONOR: As EMB-233, q.v.

RECIPIENT:- B-line female of litter 9B2/4, pregnant by litter-mate.

Inject 7 fetuses (complete complement) <sup>\*\*\*</sup> at term. Use 0.03 ml each. Injections 100% certain.

**8 JUL 1954** NINE born (see above <sup>\*\*\*</sup>): six have visible blood stains.

Note: One of the 6 with blood stain died; one of the three that at first seemed unstained had in fact a dubious discoloration. Kill the 2 definitely uninjected mice. Residue: 6, of which 5 have certainly & a sixth very probably been injected. What a blxxdy awful typewriter.

**14 JUL 1954** 5 survivors.

**22 JUL 1954** All 5 O.K.

**16 AUG 1954** TEST OPERATION. 5 mice. Donor: A 19 males.

Note: Most of these test-grafts active

26 AUG 1954

(10) B.d. complete in all grafts.

Experiment concluded.

INOCULATION OF FOETAL MICE WITH ADULT WHOLE  
BLOOD (A to CBA)

EMB-231

5 JUL 1954 Donor: As for EMB-230

Recipients: 6th litter of 1002/P. 6 embryos injected with  
0.02 ml, but at least one more present. Visualisation  
moderately good; injections almost certainly i/emb.  
Age advanced - about 18 days.

7 JUL 1954 Litter of 6 overnight - 36 hrs after injection.  
4-5 of these bear internal blood marks.

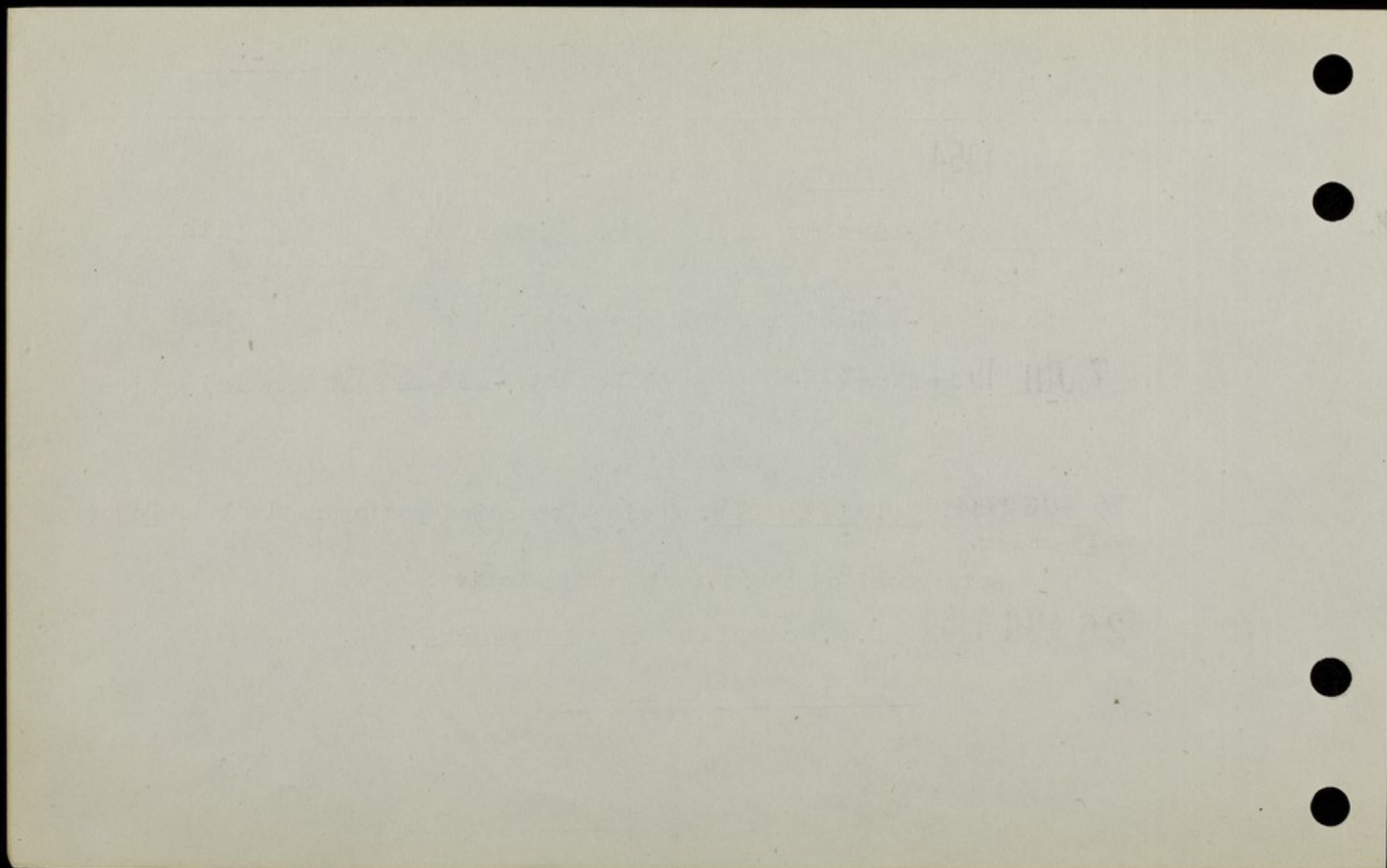
22 JUL 1954 5 mice surviving.

16 AUG 1954 TEST OPERATION. 4 mice (one died postoperatively). Donor:  
A 19 males.

Note: most of these test grafts active.

26 AUG 1954 B.d. complete in all grafts.

Experiment concluded.





INOCULATION OF FOETAL MICE (A to B) WITH  
ADULT WHOLE BLOOD

EMB-230

5 JUL 1954

Donor: Stock 16 A-line male. Citrated whole blood.

Recipient: Foetuses of female from B-line stock-pot.

6 foetuses seen and injected with 0.02 ml. Visualisation good and injections pretty reliable - chiefly i/p. Age rather advanced - about 18 days.

6 JUL 1954

Litter of 6 born overnight.

27 AUG 1954

Test-operation.

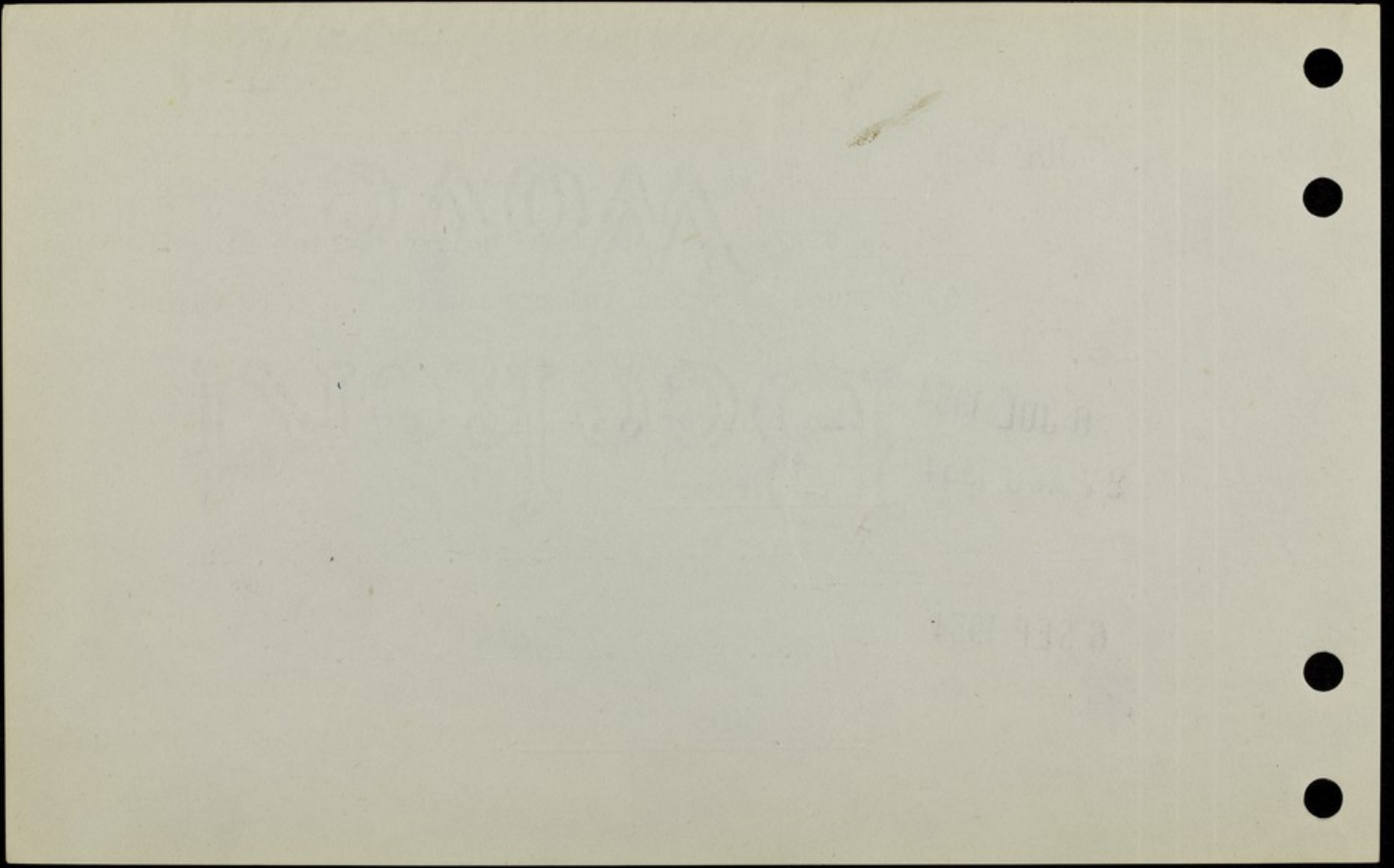
Donor: Stock 18<sup>A</sup> female. Standard grafts. RHS opns. on the 4 survivors.

6 SEP 1954

(10) B.d. complete in all 4 mice.

JUL

Experiment concluded.



28 JUN 1954 DONOR:- As 227 q.v.

-- RECIPIENT:- 10A6/2 female. 4 x 18 day fetuses (one further foetus dead). Good visualization. 0.03 ml. (Agla)

29 JUN 1954 Litter of 3 born overnight.

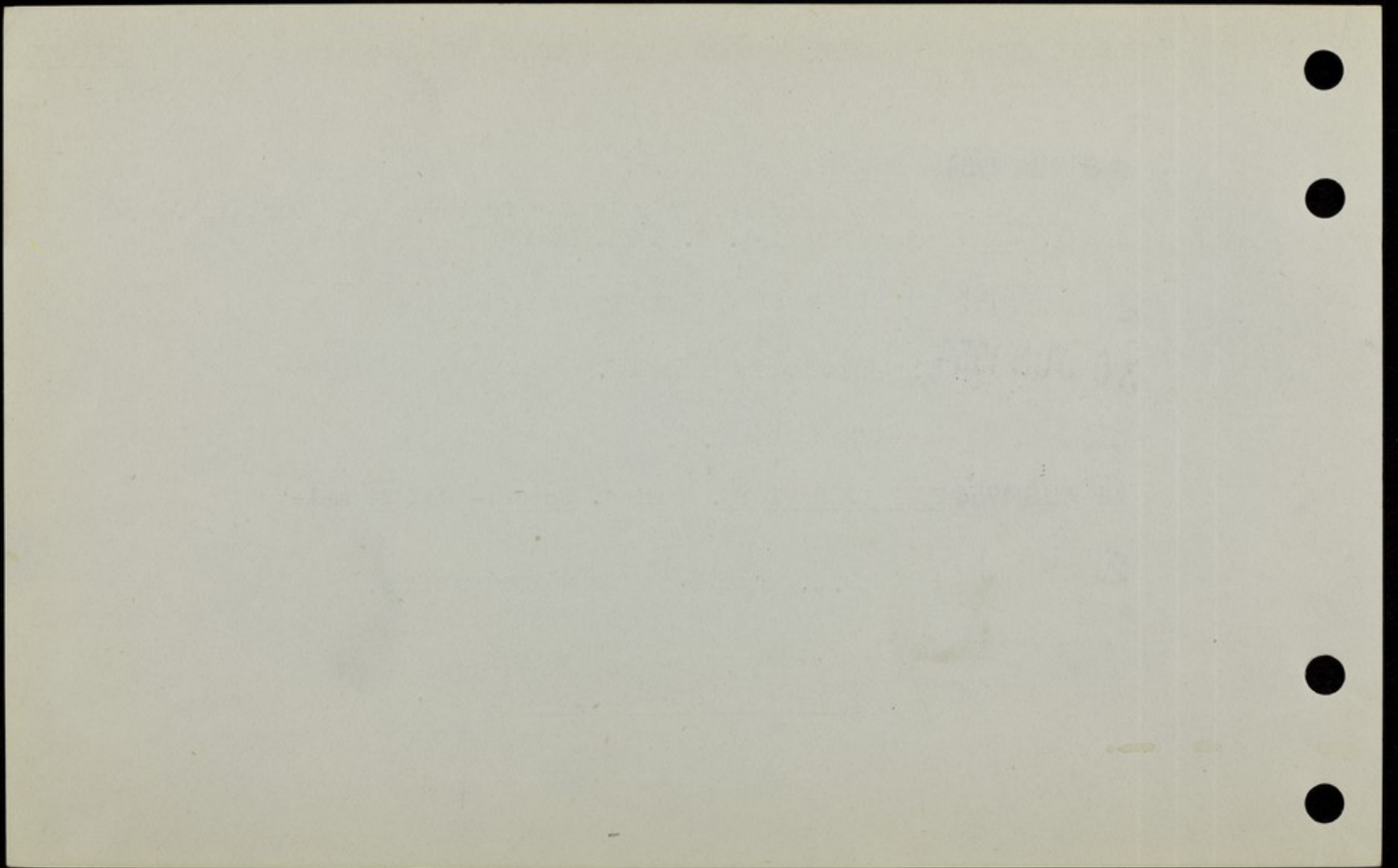
30 JUN 1954 2 survivors remain.

22 JUL 1954 Both mice O.K.

16 AUG 1954 TEST OPERATIONS. 2 mice. Donor:- CBA <sup>female</sup> 20 ~~male~~.

26 AUG 1954 (10)  
B.d. complete in both grafts.

Experiment concluded.



**22 JUN 1954** DONOR:- Stock XVII CBA male. Citrated adult whole blood.

RECIP:- O-parous F of litter 10A6/1. 7 x 15-day or less. Roughly 0.02 ml (TB syringe). Visualization bad and injections most unreliable (foetuses simply too young).

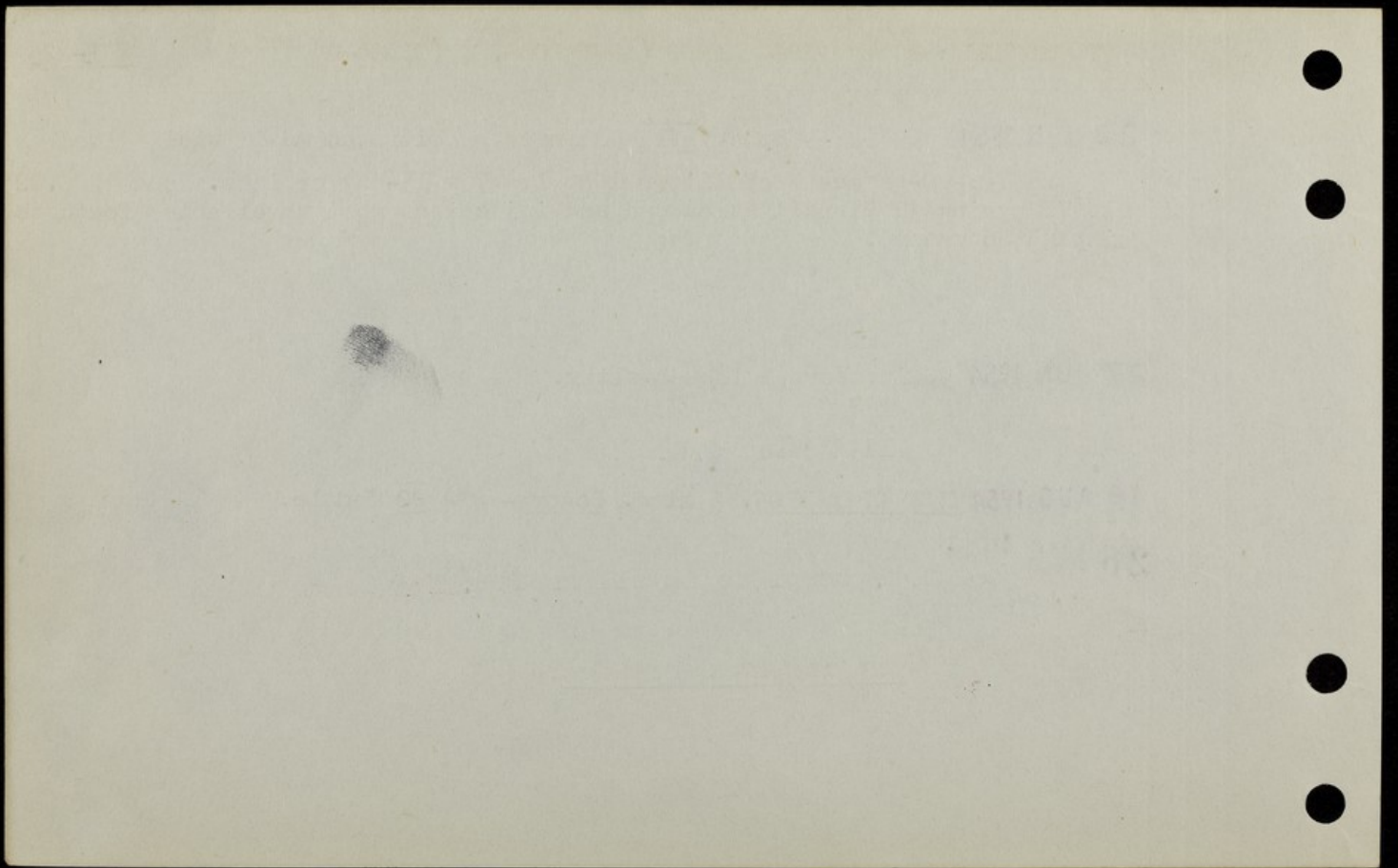
**27 JUN 1954** 5 mice born & look healthy.

**22 JUL 1954** All 5 mice O.K.

**16 AUG 1954** TEST OPERATION. 5 mice. Donor:- CBA 20 female.

**26 AUG 1954** (10)  
B.d. complete in all grafts. (cf. notes above)

Experiment concluded.



16 JUN 1954 Female from litter 8A6/7 or 8A2/9, pregnant by litter-mate; 0 or 1-parous.

7 embryos; circa 16 days, 0.05 ml; five definitely intraembryonic; other two almost certainly. Vis excellent.

DONOR:- Citrated whole blood from 2M 1F of litter 9B2/2. Citrate & siliconed tubes.

18 JUN 1954 Litter born late this evening. Age at time of injection therefore about 17 days.

20 JUN 1954 Only 2 survivors remain - these look fairly healthy.

22 JUL 1954 Both mice O.K.

16 AUG 1954 TEST OPERATION. 2 mice. Donor: B-line (AU)  $\bar{7}$  female.

26 AUG 1954  
litter.

(10) B.d. complete in both grafts. Note remnant

Experiment concluded.



INOCULATION OF FOETUSES (CBA to A) WITH ADULT WHOLE BLOOD

EMB-176

**8 MAR 1954** Donor: 7C3/p, male. Remove 1cc whole blood and citrate  
Recipients: Foetuses of female from 8A5/3, b.19 Dec. 1-parous ? L ear.  
4 + 2 --- about 17 days. Good injections except for 1 which  
was blindly injected. 0.01 cc.

12 MAR 1954

**18 MAR 1954** Healthy litter of 4 born overnight. Age at  
time of injection therefore 16-17 days.

**26 APR 1954** Test-operation.

Donor: Stock 14 CBA ♀♀ females. Recipients: 4 mice.

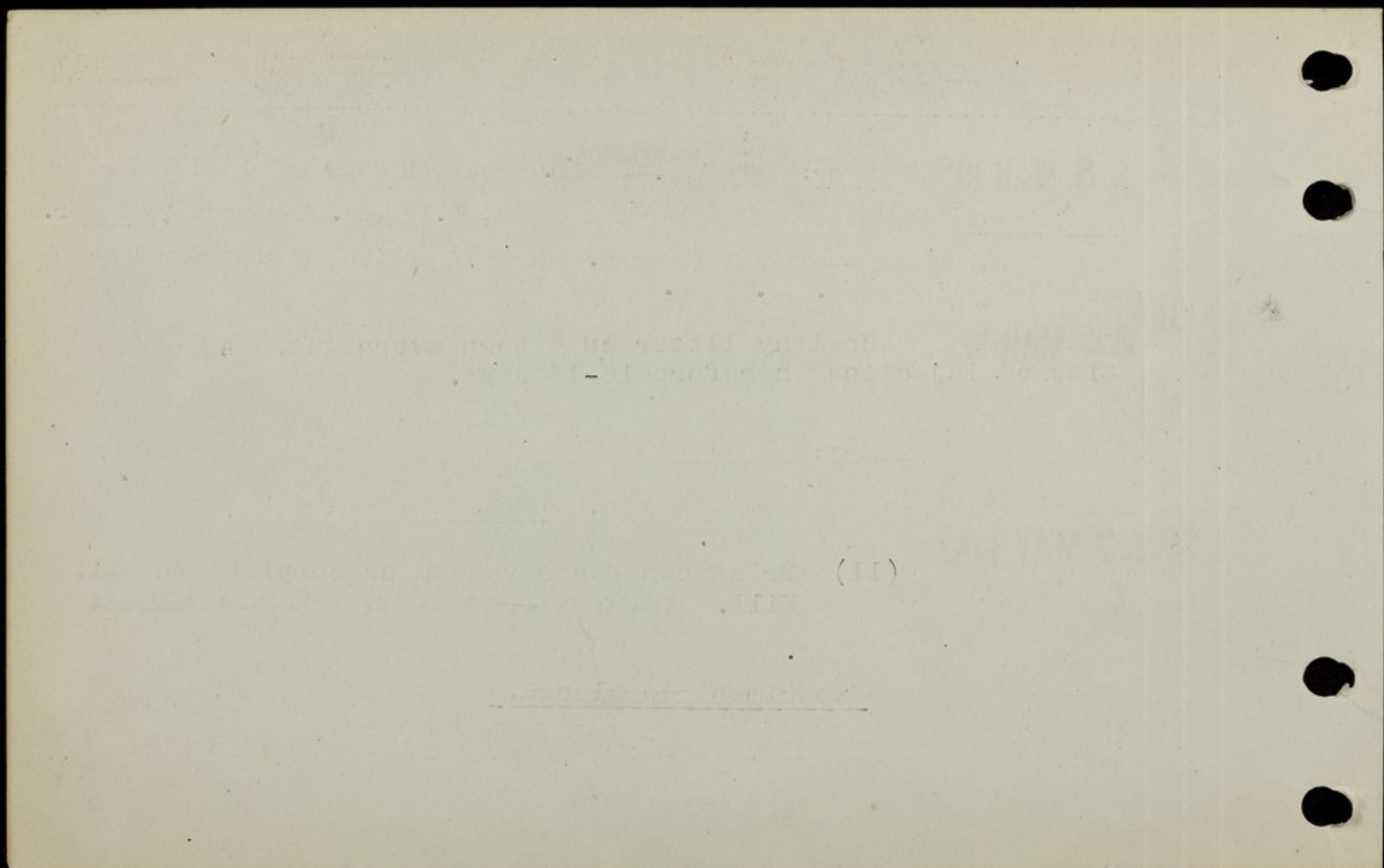
**7 MAY 1954**

(11) Breakdown far advanced or complete in all.  
Kill. *1/4 with 100% survival (S. & S. delicate)*  
*CP. 177*

Experiment concluded.

**9 MAY 1954**

(13) The only survivor has broken down  
completely. Score: 13 days. Hardly significant.



INOCULATION OF FOETAL MICE (B to CBA) WITH ADULT WHOLE BLOOD

EMB-174

**2 MAR 1954** Donor: As for EMB-172.

Recipient: Female from litter 7C9/1, b. 11 Dec., pregnant by litter-mate, probably in 1st preg. (L ear)

5 foetuses, 17-18 days old. Inject with 0.01 cc. Good visualization.

**5 MAR 1954** Litter of 7 born - 2 dead and 5 alive. The litter appears to be suckled.

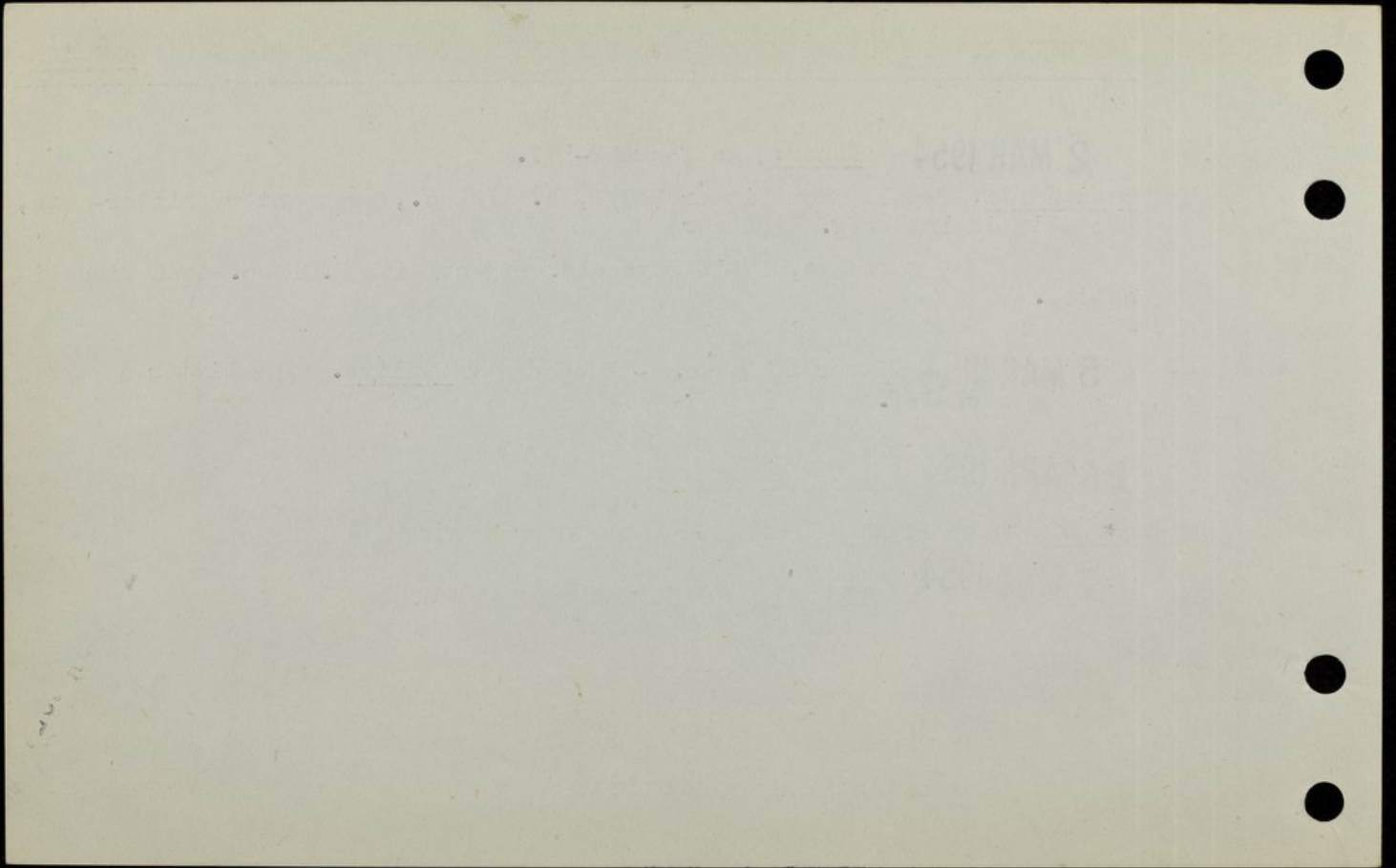
**26 APR 1954** Test-operation.

Donor: Male from 7B5/3. Recipients: 5 mice.

**7 MAY 1954** (11) All grafts broken down.

Why????

Experiment concluded.



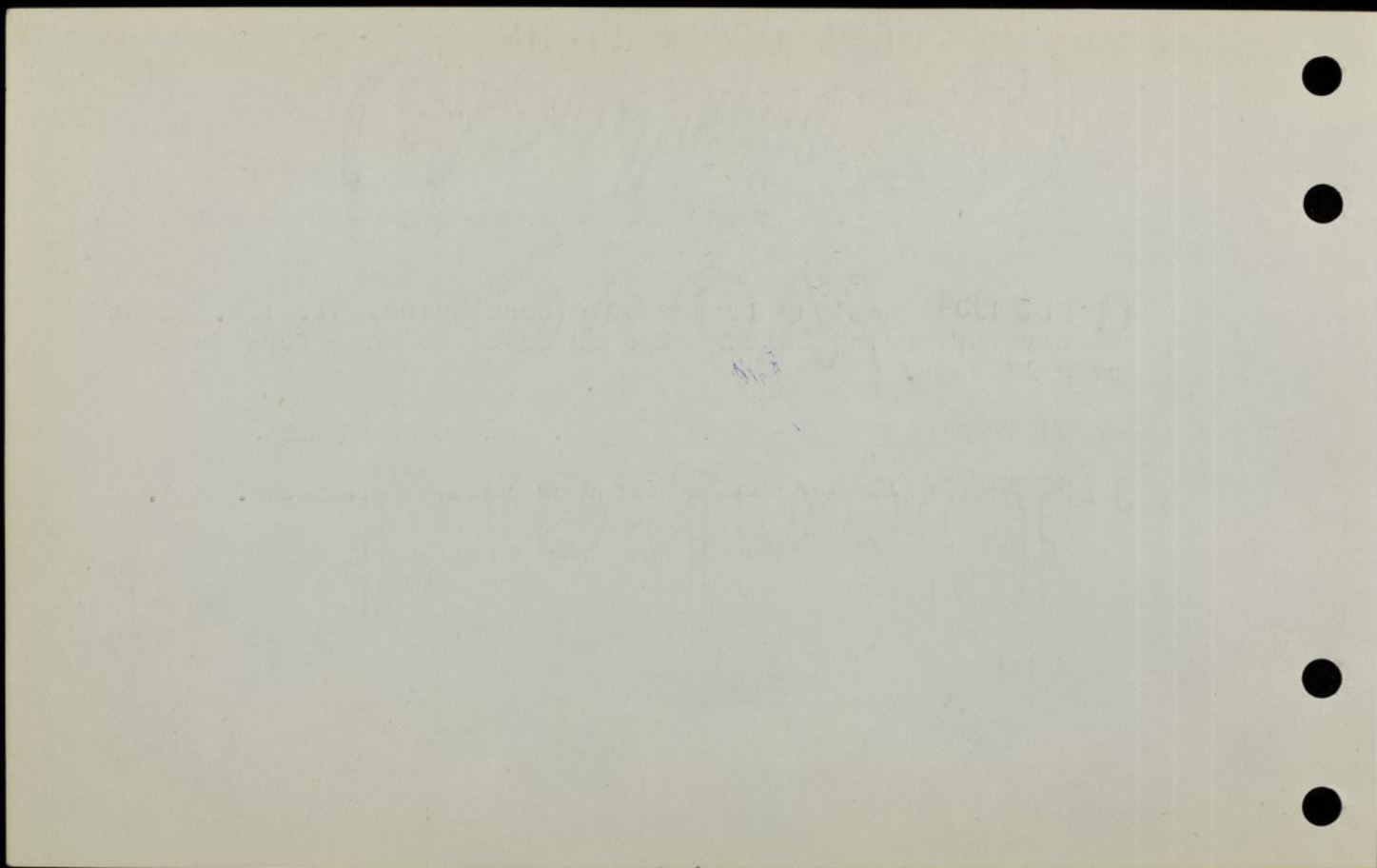
**8 FEB 1954** DONOR:- See EMB-169.

RECIP:- 8A1/4 litter female, 0-parous. See 4+2 and competently inject 17/18 day old foetuses with 0.01 mleach. R ear.

**11 FEB 1954** Healthy litter of 4 born overnight, i.e. about  $2\frac{1}{2}$  days after injection. Age of foetuses therefore just over 17 days.

**26 MAR 1954** TEST OPERATION. Donor: 8B1/3 male.

**5 APR 1954** (10) 4/4 with advanced or complete breakdown. Kill.



L notch recipient

**1 DEC 1953** Donor:- By cardiac puncture, cleanly withdraw 0.6 ml whole blood from A-line Stock VIII male. Mix at once with 0.05 ml 4% Na citrate.

Recipient:- CBA Stock V F in 2nd preg by Stock II CBA male. S.D. 40 g.

Inject 6 embryos, 16/17 day, with 0.05 ml whole blood each. Probably 7 or 8 fetuses were present. Good injections in 4/6. Visualization moderate. Age should be excellent.

Note:- Owing to a blunder, an incision about 12 mm long was made in the body wall anteriorly, & this had to be closed with closely-spaced running suture.

**4 DEC 1953** Litter of 6 (+ 1 dead) first seen this a.m. They had not been born at 6.30 p.m. last night, so that birth can be confidently timed as overnight 3/4 Dec., i.e. 2½ days after injection. Only one mouse showed residual injected blood stains clearly (but in the others there may well have been resorption).

**7<sup>th</sup> DEC:** 2 dead, 4 remain, including 1 severe emb.  
**6 JAN 1954** The four remaining mice are doing well.

13 JAN 1954 TEST OPERATION. Donor: Stock VIII A-line male. Weights 18-20g.

23 JAN 1954 (10) Very advanced b.d. in all 4 mice. No prolongation.  
Use in PT-37 as primary hosts.

Experiment concluded.

*See next page for results of 1/23/54*



**6 FEB 1954** DONOR: The male of breeding pair 7B5/P. (Kepp female for test ops). Recover 1 ml citrated whole blood by cardiac pucture.

RECIP: F of breeding pair 8A6/P, in 5th pregnancy. (Last litter 8A6/4.1-12 cp. EMB-159). 6 + 3 18-day foetuses, each 0.01 ml. Inj. of 2 foetsus not quite certain. 48g, no ear mrk.

**8 FEB 1954** Litter of 9 born overnight - about 36 hrs afetr injection.

**26 MAR 1954** TEST OPERATION. Donor: 8B1/3 male. 8 only left.

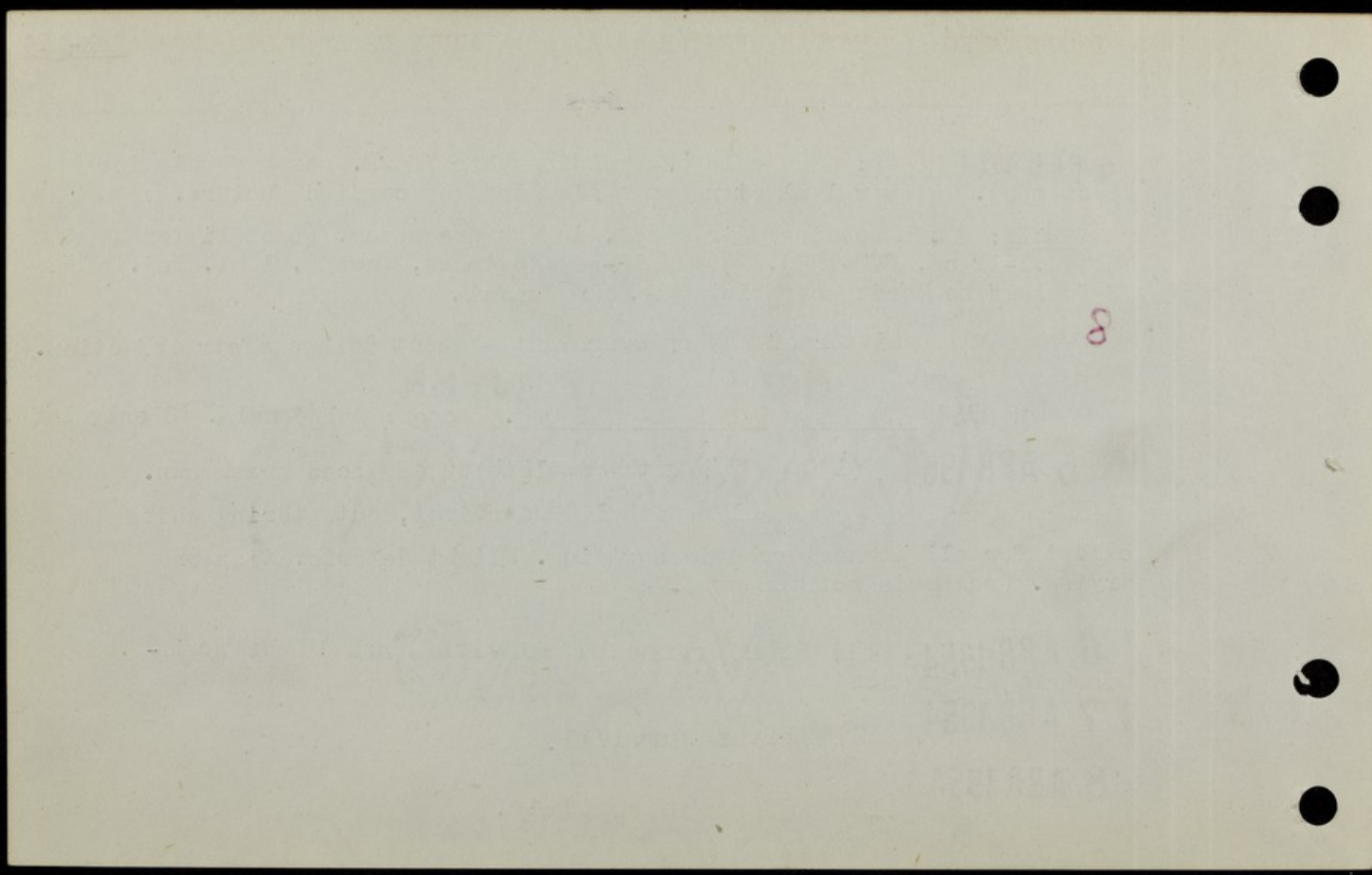
**5 APR 1954** (1)0 7/8 with complete or advanced breakdown.  
1/8 with 100% survival, but staring white

except for slight haemorrhagic spotting. Slight deterioration on drying. Prognosis not too good.

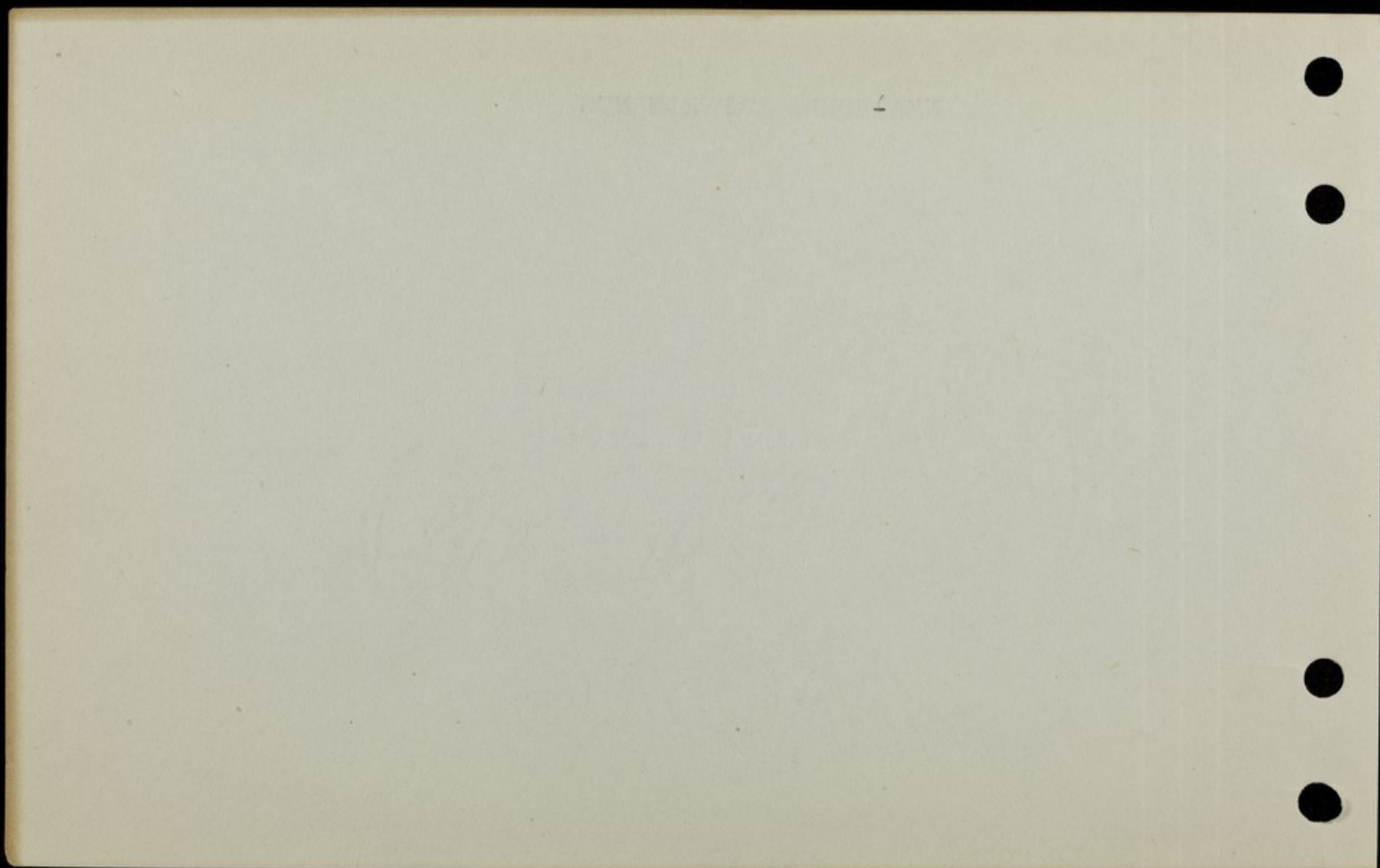
**6 APR 1954** (11) High degree of survival but light scabb  
bing in progress.

**7 APR 1954** (12) Slight survival.

**8 APR 1954** (13) Breakdown complete.



NEW-BORN EXPERIMENTS



Donor tissue: aaUU cell & cell-clump suspension exactly as used for EMB-15 q.v.

Recipients: Five newborns, 3A3/2.1-5. These had not been born at 7 on the previous night, and were injected at 11.0 a.m. this day; hence almost certainly **not more than 12 hrs old**. The litter was of 7, one having been killed and eaten, and one killed by faulty injection.

**30 JUL 1952** Each of the five newborns was given **three** injections of 0.01 ml cell suspension: RHS subcutaneously, LHS subcutaneously, and intraperitoneal. The finest (No.26 BWG) needle was used, to good effect; although fluid leaked out in some, it was much more dilute than the injected fluid, and it can be taken that most of the cells & all the larger clumps stayed in.

**2 AUG 1952** (3) All O.K.

**2 SEP 1952** All 5 O.K. Segregated.

**25 SEP 1952** TEST OPERATION.

DONOR: B- line male of 3B2/I litter, born 23 July. Remove standard full-thickness pinch grafts from flank and side, remove panniculus by scraping, and store temporarily on Ringer-moistened filter-paper. Transplant one graft to RTW scissor-clipped bed of each mouse. Ringer used to wet scissors.

I. (R), 18 g. Op. field rather anterior, avascular but no puncture,

and open fit. Mouse stopped breathing twice during bandaging, and each time the bandage had to be removed to save mouse. Op. field therefore rather messy and site was sutured. If required animal can be tested some other time.

2. F. (L), 21 g. Good fit.
3. M. (no), 24 g. Good fit.
4. M. (both), 23 g. Fairly close fit.
5. M. (R), 22 g. Fit fair.

**3 OCT 1952 (8)**

2. Healing faultless. Cuticle off with tulle. Very white and moist surface, probably little survival.  
Rebandage.
3. Healing faultless. Cuticle adherent - pull off. Graft roof haemorrhagic and pasty. Obviously no survival.  
Rebandage.
4. Healing faultless. Cuticle off with tulle. Roof slightly damp and with extensive haemorrhage. Very low degree of survival if any.  
Rebandage.
5. Healing faultless. Cuticle off with tulle. Graft roof white with slightly yellow tinge, a few haemorrhagic patches, glistening, and obviously weak. On exposure to air graft becomes scablike. Probably no survival.  
Rebandage.

**5 OCT 1952 (10)**

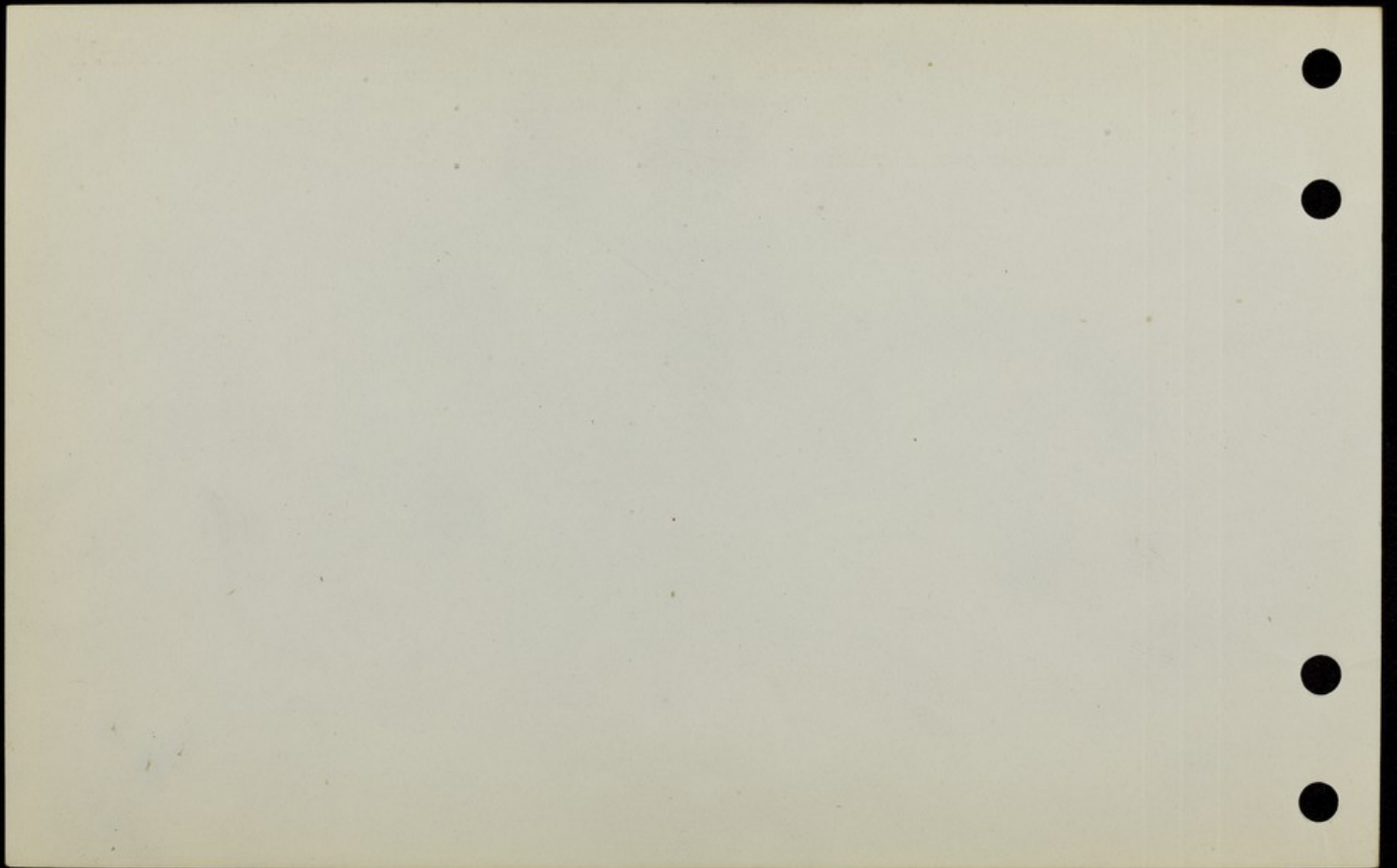
2. Graft roof very wet and white. Breakdown obviously complete. No biopsy.
3. Graft scabby. Breakdown obviously complete. No biopsy.
4. Graft roof damp, weak and with slight discolouration. At least one point

of incipient ulceration. Scabbing on exposure.

Breakdown obviously complete. No biopsy.

5. Graft is a wet and white dermal pad.

Breakdown obviously complete. No biopsy.





**28 JUL 1952** Donor:  $3\frac{1}{2}$  day old A-line mouse (still hairless), viz: 3A4/1.9. Cut strips of full thickness skin from dorsum, cut up with sharp cataract knives into tiny squares of side about  $\frac{1}{2}$  mm, and load three to five of these into a hypodermic-trochar (smaller than that used for EMB-12,13) for injection into each mouse.

Recipients: Litter of 6 from non-line multiparous cba female, b. March 3rd, total weight 7.26 g., mean weight 1.21 g. Age not more than 2 hrs post partum (delivery started at 4 p.m., injections completed by 6.15 p.m.).

Inoculate each by needle insertion in R groin passing forwards to RTW area; then inject each mouse over left shoulder region with 0.05 mg cortisone (0.002 ml standard cortisone suspension containing 25 mg/ml).

**30 JUL 1952** (2) Total wt. 9.23 g., mean wt. 1.54 g. Grafts visible in all; virtually no body pigmentation. Reinject with 0.05 mg cortisone each.

**1 AUG 1952** (4) Total wt. 11.20 g., mean wt. 1.86 g. Litter healthy but retarded. One is particularly backward in general development and may have to be written off sooner or later. Retardation does not seem as pronounced as ~~at~~ in EMB-13 at same age - note difference in mean wt.

Grafts visible in all but positions vary somewhat. Impression is they are attached. Remnant of cortisone visible in all. Eyes and ears are well pigmented. Pigmentation of dorsum of thorax (on either side

of backbone) has begun, but posteriorly there is only a very faint trace.  
Inject as above with 0.05 mg. each. (With the exception of the runt).

2 AUG 1952 (5) All O.K., but looking rather weak & blanched

4 AUG 1952 (7) Pigmentation still very backward & animals undersized, but not emaciated and moderately lively.

6 AUG 1952 (9) a.) 3 very healthy and lively, but backward. Well-marked pigmentation and hairgrowth on snout and dorsum of thorax. Rest of body still pale except for ears, eyes, tails and distal limbs. Vibrissae well developed. Eyes still closed.

b. 1 very small and particularly backward - probably destined to die. Hardly any pigmentation and no hairgrowth.

c. Intermediate. (1)

Grafts visible in all, though pigmentation and increase in thickness of skin are beginning to obscure their location.

Note that one mouse has disappeared (presumably it died and was then eaten). 5 remain. Total wt. 12.9g. Mean wt. 2.58g.

12 AUG 1952 Eyes open according to normal practice - no retardation.

25 AUG 1952 The runt has died as expected. The remaining 4 are fairly healthy, but still very small.

25 SEP 1952

TEST OPERATION.

EMB-I4

DONOR: A-line male of 2A4/2 litter, born II JUNE. Remove and transplant to each mouse in exactly the same way as for EMB-I2, but to RTW.

1. M. (R), 20 g. Close fit.
2. M. (L), 21 g. Excellent fit.
3. M. (no), 19 g. Close fit.
4. M. (both), 23 g. Very close fit. Dorsal puncture.

28 OCT 1952 (8)

1. Healing faultless. Cuticle off with tulle. Graft roof covered with film of serous exudate and with extensive haemorrhage. Slight puffiness. Probably little epithelial survival. Rebandage.
2. Healing satisfactory. Cuticle off with tulle. Graft roof with dry, supple, epithelialised surface and pinkish tinge. A few red points are visible - so small that they could be non-specific. Rebandage.
3. Healing faultless. Cuticle off with tulle. Graft slightly puffy. One or two small haemorrhagic patches, but on the whole graft delicately pink and gives healthy impression. Rebandage.
4. Healing faultless. Cuticle off with tulle. Roof damp and with haemorrhagic patches of some severity. Degree of survival probably low. Rebandage.

7 OCT 1952 (12)

1. Graft roof is fairly dry and has a not unhealthy colour. Possibly some epithelial survival.  
Specimen EMB-I4-1. Strip with host skin at each end. Formol/Hg.
2. Graft roof is dry and white and appears to be epithelialised, but by no means autograft-like. Graft uncontracted. Probably fair survival.  
Specimen EMB-I4-2. Strip with host skin at each end. Formol/Hg.
3. As for 2 but with dorsal patch of faint redness.  
Specimen EMB-I4-3. Strip with host skin at each end. Formol/Hg.
4. Graft very damp and with slight discolouration. Obviously nothing but a dermal pad remains. No biopsy.

#### Histology.

1. 5% survival.  
There appears to be some trace-survival, but it is difficult to separate this completely from native ingrowth which is clearly taking place. Intense round cell reaction in graft bed.
2. Breakdown complete.
3. 5% survival.  
Only a few nests of indolent follicles survive.

**NOTE:** There is here a complete lack of an immune effect.

24 JUL 1952 Donor: 2AX/1.5, 60 <sup>hr</sup> ~~day~~ old approx A-line newborn. Cut unsoap-washed skin into micrografts each about  $1\frac{1}{2}$  mm square; load 2 or 3 fragments into tumour implantation trochar and implant subcutaneously by groin insertion passing forwards under skin of standard op. field on RHS thorax.

Recipients: 4 aaUU embryos, approx. 8 hrs after birth; born of either sib or cousin mating of female of litter 2B6/1, b.1 May 1952.

26 JUL 1952 (2) All 4 babies doing well; one is runtish. Gross weight 6.31 g, mean weight 1.58 g. Implants are visible in 3 out of 4; mark the one in which they are not visible by curtailing. All implants have moved backwards or dorsally from original implantation site. Trochar insertion points well healed.

Except for the one backward baby, all show a well-defined dorsal shadow of pigmentation.

28 JUL 1952 (4) The backward baby is catching up. All now have full deep pigmentation over entire back. Total weight 10.02 g., mean 2.51 g.

30 JUL 1952 (6) Graft is now visible in curtailed specimen (see day 2). Graft is unduly mobile in one animal. Hair growth has started; deep normal skin pigmentation. Wt. 13.30 g., mean weight 3.33 g.

2 AUG 1952 (9) These are now at the puppy-stage, plump and with fine even belt of black hair.

2 5 AUG 1952 The sole male has been segregated to avoid pregnancy.

5 SEP 1952 One female young has died. 2 females and 1 male are now left.

2 5 SEP 1952 TEST OPERATION.

DONOR: A-line male of 2A4/2 litter, born 11 June. Remove standard full-thickness pinch grafts from flank and side, trim off panniculus by scraping, and store temporarily on Ringer-moistened filter-paper. Transplant one graft to LTV scissor-clipped bed of each mouse. Dressing as usual. Ringer used to wet scissors.

1. F. (R), 21 g. Fair fit.

2. F. (L), 20 g. Rather loose fit, and bed unusually avascular.

Note: A small whitish palpable body was observed below the skin of RTW. From it protruded a very small tuft of white hairs. Locality is definitely that of the implanted micro-grafts.

3. M. (no), 23 g. Excellent fit. Palpable nodule on RTW.

3 OCT 1952 (8)

1. Healing satisfactory. Cuticle off with tulle. Graft roof very moist, white and with severe haemorrhage in dorsal half. Survival unlikely.  
Rebandage.

2. Healing faultless. Cuticle off with tulle. Roof dry and pinkish. Some points of haemorrhage. Epithelial survival probably fair. Rebandage.
3. Healing satisfactory. Cuticle pulled off. Graft swollen and with damp, mushy, sponge-like appearance. Low degree of survival if any. Rebandage.

5 OCT 1957 (10)

1. Graft partly covered by cuticle - pull off. Roof very wet and white. Breakdown pretty obviously 100%.  
Specimen EMB-12-1. Strip with host skin at each end. Dormol/Hg.  
Note: No organised graft could be found in a subcutaneous position on the RTW.
2. Pull off cuticle. Wet, haemorrhagic and discoloured surface. Survival almost inconceivable.  
Specimen EMB-12-2. Strip with host skin at each end. Formol/Hg.  
Note: A small white pad was found to adhere to the internal side of the panniculus of the RTW. A small tuft of white hairs protruded from it through the skin. BIOPSY and section. Spec. EMB-1262-G.
3. Graft roof is a moist, pasty, discoloured mess, with several points of open ulceration. Breakdown complete.

Histology.

12.2. Breakdown complete.

12.2.G. (Subcut. cyst)

Cyst in excellent condition and without the slightest sign of a ~~reaction~~ reaction. Epidermis towards the middle, follicles and sebaceous gland tissue in the surrounding cyst dermis. Cavity of cyst (which is clearly skin) filled with hairs and cuticular debris. No sign of a round cell infiltration.

The cyst is homologous, and most of the epithelium is nonpigmented. There is however a small patch of pigmented epithelium on the S side of section 2. Could the melanocytes have invaded the foreign epithelium from neighbouring host follicles???

The complete survival of this cyst, accompanied by the normal breakdown of the animals test-graft ~~remains~~ remains unexplained.



**16 AUG 1952** DONOR TISSUE: Exactly as EMB-18 q.v., except that a fresh suspension was prepared from intact tissue lumps stored 48 hrs at about 4 deg.C (see EMB-18).

RECIPIENTS: Litter of 7 from aaUU female of litter 1B/1 (= 2B1/P), b. 17 Feb 1952, multiparous. Inject under left & rought shoulders subcutaneously and intraperitoneally: 0.01 ml of a dense cell + cell clump suspension in each position. No cortisone. Age of litter: Had not been born at 6 p.m. the previous night, injected 10 a.m., hence maximum age 16 hrs, probable age about 8 hrs.

One baby was punctured through stomach in intraperitoneal injection.

**18 AUG 1952** One of these babies inadvertently injected with 0.05 mg cortisone. Mark this baby by curtailment.

**8 SEP 1952** 1 young mouse dead. It is the ~~2nd~~ baby which was accidentally injected with cortisone - surely a glorious example of the survival of the fittest!

**10 SEP 1952** Another death. This mouse must have died several days ago and was found buried in the saw-dust.  
5 remain.

~~**11 SEP 1952** Yet another death. Cage sterilised.~~

**19 SEP 1952** Males and females segregated together with those of EMB-20

21 OCT 1952

Test operation.

DONOR: 2A2/I, female, 31 g. Operation as for EMB-25.

1. F. (R) 19 g. Close fit.
2. F. (L) 19 g. Open fit. Small ventral puncture.
3. F. (no) 18 g. V. close fit.
4. M. (both) 24 g. Fit fair.
5. M. (2R) 21 g. Close fit.

29 OCT 1952 (8)

1. Cuticle off with tulle. Healing faultless. Surface wet and haemorrhagic, and partial loss of epithelium. Probably little survival. Rebandage.
2. Healing faultless. Cuticle off with tulle. As for 1. Rebandage.
3. Healing faultless. Cuticle off with tulle. Similar to 1, possibly slightly better. Bandage.
4. Healing faultless. Cuticle off with tulle. Surface looks weakish, has two haemorrhagic patches and a superficial yellowish discolouration. Some survival possible. Rebandage.
5. Healing faultless. Cuticle off with tulle. Considerable swelling and intensified haemorrhage - most of graft deep red. Survival of some ventrally situated epithelium possible. Rebandage.

31 OCT 1952

EMB#24

~~31 OCT 1952~~ (100)

1. Moist graft surface but it looks almost better than at first inspection. (This may well be due to disappearance of haemorrhagic pattern). On exposure to air large area of central reddening. Probably little or no survival.

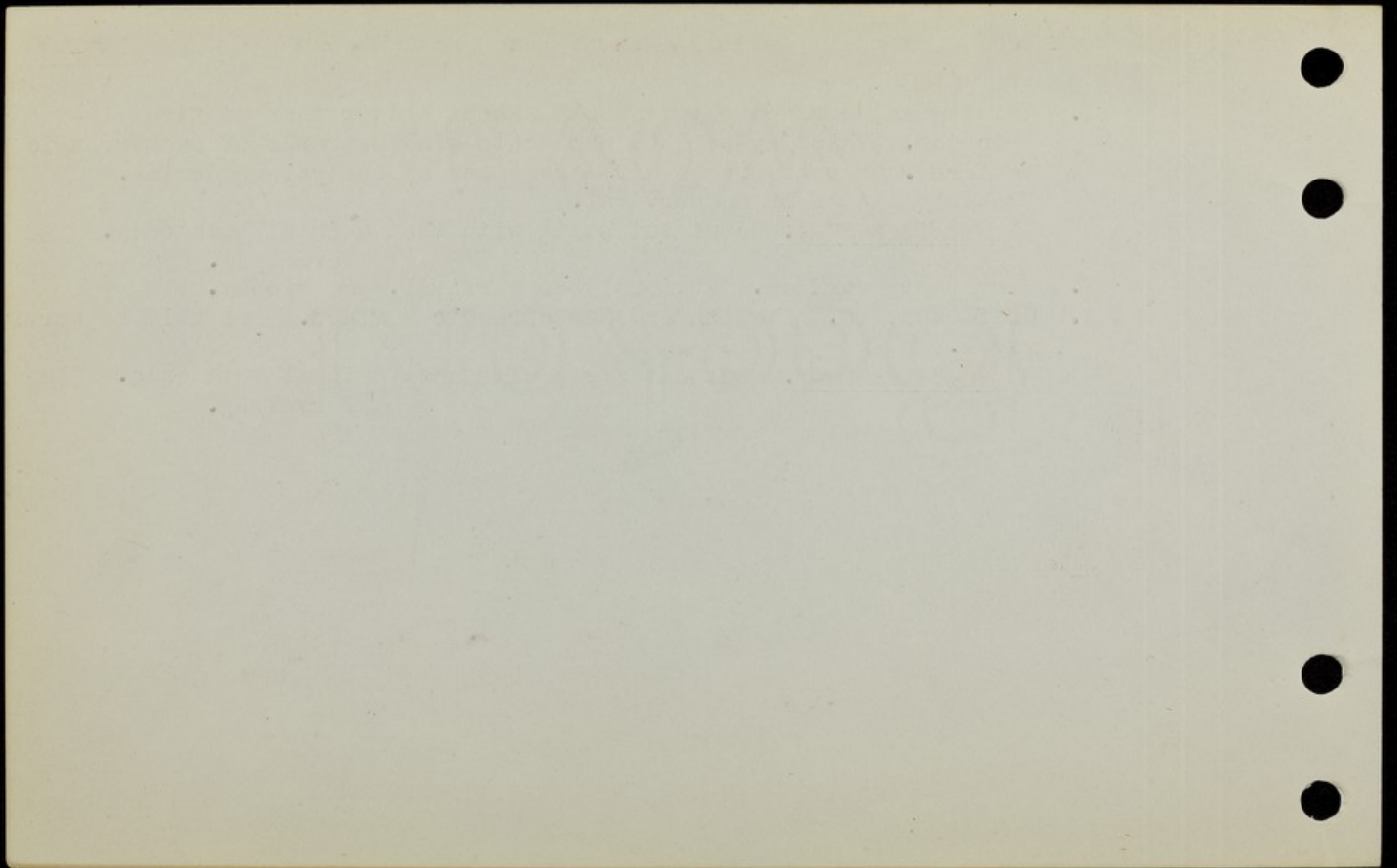
Specimen 24-1. Cut out strip with host skin at both ends.  
Formol/Hg.

2. Very pasty surface. Obviously no survival. No biopsy.

3. Graft swollen. Dampish and haemorrhagic surface. Probably no survival.

Specimen 24-3. Cut out strip with host skin at both ends.  
Formol/Hg.

4 & 5: Survival highly improbable.  
No biopsies.



**26 AUG 1952** Donor tissue: Skin from dorsum of 5-day old A-line mouse, free from panniculus. Chop up into minute squares of about  $\frac{1}{2}$  mm length of side, and inoculate four to six of these squares through a medium thick hypodermic syringe fitted with a plunger.

Recipients: The first litter, of six, from female Z-1 (itself of litter 2C2/1, b.22 June, and pregnant by a litter mate). The litter had not been born at 10 a.m. this day, and when first seen at about 4 p.m. was still imperfectly cleaned up. Cannot be more than 6, more probably 2-3 hours old.

Each newborn was inoculated subcutaneously with 4-6 skin grafts in the R op.field region. Little deposits of implanted tissue visible in all.

**31 AUG 1952** All 6 O.K.

**9 SEP 1952** One baby dead - post-mortem and examination for graft not possible.(due to mauling). 5 remain.

**21 OCT 1952** Test operation.

DONOR: 2A2/I female, 31 g. Operation as for EMB-25. BUT LTW.

1. F. (R) 17 g. Close fit. (RTW)
2. F. (L) 17 g. Close fit.
3. F. (no) 16 g. Close fit.
4. M. (both) 20 g. Close fit.
5. M. (2R) 20 g. Close fit.

29 OCT 1952 (8)

1. Healing faultless. Cuticle off with tulle. Roof very wet and showing a very clear-cut dermal pattern. Epithelial survival likely to be low. Rebandage.
2. Healing faultless. Cuticle off with tulle. Graft dry, epithelialised, and pink, but some inflammation ventrally and tendency for epidermis to peel off. Rebandage.
3. Healing faultless. Cuticle adherent - its removal reveals moist surface. Rebandage.
4. Healing faultless. Cuticle mainly off with tulle. Roof healthy pink, but dampish, slightly swollen, and with incipient haemorrhage. Rebandage.
5. Healing faultless. Cuticle off with tulle. Roof dampish and with incipient haemorrhage, Rebandage.

3 NOV 1952 (12)

1. Graft roof pasty and discoloured. No survival. No biopsy.
2. Roof pasty and ulcerated. No survival. No biopsy.
3. Roof moist and with several points of ulceration. No survival. No biopsy.
4. Pull off thin cuticle. Roof moist and white with redness in centre. Dermal pattern conspicuous. Survival unlikely.  
Specimen EMB-3I-4. Cut out strip with host skin at both ends.  
Formol/Hg.
5. Roof wet, discoloured and haemorrhagic. No survival. No biopsy.

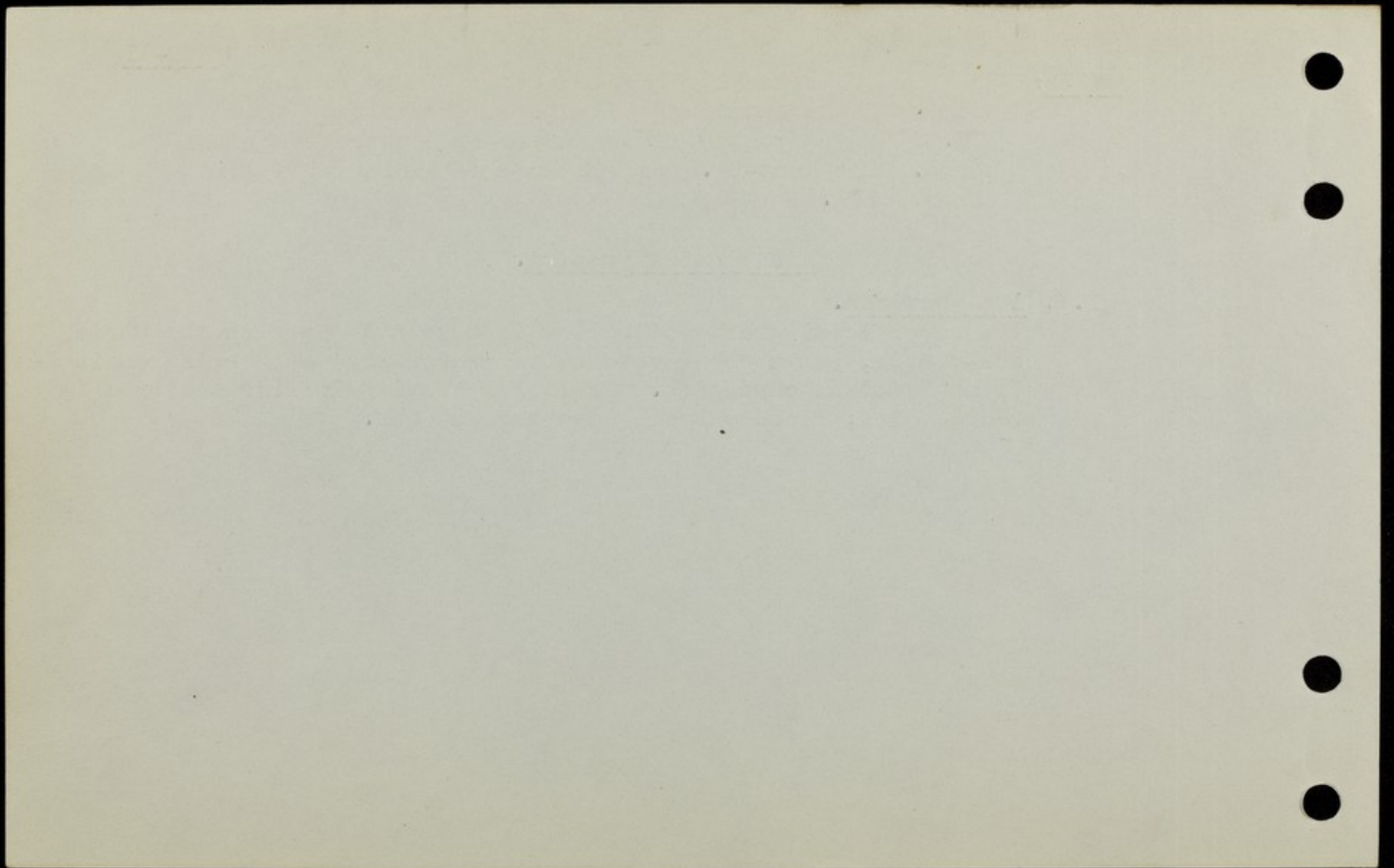
P.T.O.

NOTE: In none of these animals was a trace found of the primary "desensitising" subcutaneous grafts on the RTW. It appears that these have been destroyed by the hosts. The test-op. results are in keeping with this observation.

Histological report.

31.8. 50% survival.

The superficial graft epithelium is seperating and on the whole non-viable, but a few stretches of 'secondary' epidermis remain in an indolent condition. Graft bed heavily infiltrated by round cells; blood vessels have broken down.





1 NOV 1954 Donor tissue. Remove 2 kidneys, 2 testes, spleen and spleen-sized bit of liver from 60-day old cba mouse of litter 2C2/3, b.2 Sept. Cut spleen, liver into two; transfer all at once to warm Ringer. Store  $\frac{1}{2}$  spleen,  $\frac{1}{2}$  liver fragment, 1 testis, 1 kidney on Ringer-wetted filter paper in Petri dish until next injection (temp. about 5 deg.C.). Grind up the rest with scissors for about 30' under Ringer, almost the whole being reduced to very small tissue fragments passing easily into No.26 needle. After passage through needle, spin down cells in flat-running centrifuge to a compact mass. Reject most of the supernatant, and take deposit up in the remainder to form a really concentrated brei of more than thick creamy-viscosity. Some red cell present (reddish-brown tinge). In future avoid this by cutting into coarse lumps first and washing in Ringer. The brei went back fairly easily through the 26 BWG needle.

Recipients. Litter 3A2/5, which was being born at 1.15 p.m., and on which the injections were completed by 3.30 p.m., so that maximum age  $2\frac{1}{2}$  hours. Further, as the litter was large (8) it is probable that birth was relatively premature. Of these 8, one was a 'blue mouse' and was destroyed. The other seven injected as below.

Mount the Agla very firmly in a retort clamp and keep it fixed at an appropriate height. Anaesthetize each mouse by chilling on a cold-plate, and do all injections by manipulating the almost completely torpid mouse inside of the syringe. Administer 0.01 ml on each side of chest subcutaneously, and 0.01 ml intraperitoneally. Virtually no loss from subcutaneous sites, and only trivial loss from i/p sites. Whole session v. successful.

3 NOV 1952 (2)

Each young mouse reinjected with 0.01 cc subcutaneously on each side and intraperitoneally. Method exactly the same as before, including the preparation of the cell suspension which consisted of a brei of cell-clumps. Brei prepared from stored material.

Litter in good condition - one mouse is slightly runtish.

5 NOV 1952 (4)

Litter reinjected. Each mouse received 0.01 cc on each side of thorax (subcutaneously) and 0.01 cc intraperitoneally. Method of injection as before (clamped syringe).

Donor tissue: male cba 2634, born 31 August. Remove 2 kidneys, 2 testes, spleen and spleen-sized piece of liver. **STORE HALF**. Thick brei prepared exactly the same as before from the other half.

Suggestion for the elimination of red cells at early stage of chopping up (see 1 Sept.) is not practicable since the red cells are not liberated until later in the operation.

**Injections** very successful. Litter in good shape, including the runt.

8 NOV 1952 (7)

Each mouse reinjected with 0.01 cc subcutaneously on each side of thorax and 0.01 cc intraperitoneally. Method of injection exactly the same as before, and very successful.

Brei prepared from stored tissue from 2C3/4. Method of preparation exactly as before.

Litter in excellent shape.

07 NOV 1952 (10)

Each mouse reinjected with 0.01 cc subcutaneously on each side of thorax and 0.015 cc intraperitoneally. Method of injection as usual. Litter in good shape and injections successful.

DONOR TISSUE: Remove from 2C3/4, b. 31 August, both testis, both kidneys, spleen and half a lobe of the liver. Store half of these tissues in frig. From rest prepare thick brei exactly as before.

14 NOV 1952 (13) Prepare fresh brei from the tissue stored since 11 Nov. Into each mouse (now with pelt of hair) inject 0.1 ml on each side s/c and 0.2 ml i/p. Two mice were injected twice with this trio of injections because of a doubt about whether the first trio had in fact gone in (loosening of plunger). These two may therefore have received somewhat more than the dose given to the other five,

19 NOV 1952 (18)

Prepare fresh brei from tissues of new donor, and re-inject.

DONOR: "Non-line" cba male, from litter of Y-2, b. 17 August. Remove both testes, both kidneys, half a lobe of liver and the spleen. Store half of these tissues at -5 deg. for future injection. From rest prepare brei using the now well-tried technique.

Inject each mouse subcutaneously on each side of thorax with 0.03 cc. and intraperitoneally with 0.03 cc. One mouse, distinguished from the others by a left ear notch, received 0.1 cc intraperitoneally. Injections satisfactory. A coarse needle was used.

24 NOV 1952

Litter accidentally injected with cell brei prepared from another A-line mouse. (L.B. takes full responsibility). Whilst no harm has been done the animals cannot be re-injected with homologous cells for at least 2 days.

26 NOV 1952

Donor tissue: Half of spleen, small piece of liver, and one kidney taken from female cba 3C2/1, b.28 August. A dense brei was obtained by usual technique.

In ject each mouse as follows: 0.03 cc subcutaneously in mid-dorsal line, and 0.04 cc intraperitoneally.

Injections very successful.

Note: The donor organs had been removed from donor on previous day and stored at -5 deg.

27 DEC 1952

(30) Donor tissue: Whole spleen, one kidney, and piece of liver approx. the size of the spleen. These organs were freshly removed from pregnant cba female 23C/4, b. 31 August. The brei was prepared in the usual way and was exceedingly concentrated. Re-inject each mouse as follows: 0.04 cc intraperitoneally, and 0.02 cc subcutaneously on each side of thorax.

5 DEC 1952

(34) Donor tissue: Piece of liver,  $\frac{1}{2}$  spleen & whole kidney from new donor, female of litter 2C2/3 b.2 Sept (pregnant within line: see EMB-63). Scissors method, very dense brei, wider bore needle. 0.03 ml i/p and into each side s/c.

11 DEC 1952

(40) Donor tissue: Piece of liver, whole spleen, one kidney, and both testes from male of litter 3C2/1, b.28 Aug. Scissors method, very dense brei, coarse needle. Inject 0.03 subcutaneously in mid-dorsal line, and 0.03 cc intraperitoneally. EMB\*57

23 DEC 1952 (52) TEST OPERATIONS. Donor: Cut & trim 7 body skin grafts from 3C1/P male; transplant one each to the seven below. Dorsally the skin of this mouse was active & in a cycle of hair growth; this may prolong survival detectably if the wave of activity has already spread to the lateral & ventro-lateral areas from which grafts were cut.

Recipients:- 57:1-7, using full nembutal anaesthesia & scissor-clipping method throughout. Note that the females were far more refractory to Nembutal than the males, and supplementary ether had to be used in all.

57.1 22½ g. L ear. M

57.2 23½ g. R ear. M. Very thick, active skin on graft bed.

57.3 22½ g. No mark. M. Dorso-lateral approach.

57.4 19½ g. L ear. F. )

57.5 19 g. R ear. F. )

57.6 19 g. Both F. )

57.7 18 g. No mark. F. )

Dorso-lateral approach<sup>++</sup>. Exceedingly thin skin, but no pictures.

All plasters picrated. <sup>++</sup>Dorso-lateral approach; mouse tied by a single rubber noose round both forelimbs, but separate leg ties, so that the lie is ventral posteriorly. The grafts lie dorsal to the main lateral vessel group. This technique is a decided improvement for manipulation.

**30 DEC 1952** (52 + 7) Test inspection of sample. Three mice chosen at random: 1,5,6. Etherize; and after inspection vaseline the graft & replaster.

- (1) Graft perfectly healed; very decidedly haemorrhagic, with rather damp surface.
- (5) Graft perfectly healed; clean separation of ghost. Some vascular dilatation centrally, and generally appearance not too good. However, certainly not abject breakdown as if mouse had been immunized.
- (6) Graft perfectly healed; ghost separated cleanly. Seems O.K.

**2 JAN 1953** (52 + 10) Complete inspection. In general, the appearances are of CBA to A grafts on normal untreated animals. These gave an MST of  $10.4 \pm 0.3$  days; at 10 days, 3/8 showed total breakdown. In the present group, to outward appearance, 5/7 seem to show very advanced breakdown, so there may be some immunization effect.

- 57.1 See 52 + 7 days. Abject necrosis of very long standing, the graft surface being totally eroded & now infected.
- 57.2 Big graft; white & with punctate haemorrhages. Epidermis peels off to reveal moist surface. Breakdown in progress or complete.
- 57.3 Wet reddened surface (slit in L ear posteriorly; accidental). Breakdown complete or nearly so.
- 57.4 Graft looks whitish & irregular, but not haemorrhagic, and surface dry.

- 57.5 Graft white; epidermis could be peeled off to reveal wet surface. Transparent appearance. Breakdown total or nearly total.
- 57.6 Graft looks perfectly O.K. except for being a bit white; it is unswollen, supple and firm. **DO NOT KILL**; apply germolene without other dressings.
- 57.7 Ghost off as a sort of serous + cuticular scab, in one thick plate. The surface so revealed is moist and very haemorrhagic, with the most unusual appearance of free bleeding. Breakdown probably complete.

**REMOVE** all grafts except 57.1 and 57.6 for histological examination.

After removal of biopsies a search was made for the remains of injected material:

2. Graft rather curled up on the filter-paper. Hardly any trace of injected material subcutaneously - only two tiny masses, light brown in colour and loosely attached to connective tissue. Small whitish masses, hardish and almost calcareous in appearance, found free in body-cavity. All material removed and fixed. Spec. 57.2.R
3. 2 or 3 small pigmented masses attached to connective subcut. near graft. Whitish and quite large masses in body-cavity - some completely free, some loosely attached to connective of liver, intestine or body-wall. Fix. Spec. 57.3.I<sub>n</sub>.

4. Scar now in centre of graft. A few small strands of lightly pigmented tissue attached to connective of skin on side opposite to that of graft. A number of free greyish, hard masses in body-cavity, a few loosely attached. Fix in Formol/Hg. Spec. 57.4. In.
5. Same as 7. Fix in Formol/Hg. Spec. 57.5. In.
7. Small light brownish strands attached to connective tissue subcut. Greyish, fairly hard lumps in body-cavity (free). Fix. Spec. 57.7. In.

**3 JAN 1953** (11)

6. Graft whitish, uncontracted and epithelialised. Survival seems to be complete.

**4 JAN 1953** (12)

6. Small ventral scar which may foreshadow breakdown. Rest of graft is white and appears to be epithelialised. The scar has the appearance of a serous scab and therefore is unlikely to be due to scratching.

**5 JAN 1953** (13)

6. Whole of ventral half now scabbed. Dorsal half white, apparently epithelialised but swollen. Breakdown seems to be well under way.

**6 JAN 1953** (14)

6. Whole graft now scabby, severely contracted and generally appearance of B.D.L.S. Slight white rim remains dorsally - this is probably due to overgrowth. Specimen EMB-57.6. Fix in Formol/Hg.

NOTE; The behaviour of this graft has not turned out to be as anomalous as was at first expected. Breakdown was probably complete by 13 days.



Search for injected material resulted in same observations as for EMB-57.7. Fix. Specimen EMB-57.6.In.

Histological Report.

*1. B.D. complete - Ho 500 p. 7.*

2. 75% survival. (10)

Epithelium predominantly viable except for a narrow strip running right across graft. Vascularisation is bad and proliferation very subdued. Capillaries are chiefly intact, only limited haemorrhage. Cellular reaction, such as it is, is in the graft bed - infiltration into the graft is very moderate.

3. Breakdown complete.

4. 25% survival.

Breakdown practically complete over 2/3 of the graft, but rest shows fair survival.

5. 5% survival. (Follicular trace.)

6. Breakdown complete. (BDLS)(14)

Overgrowth and undermining in progress.

7. Breakdown complete.

CONCLUSION: 3/6 survivors at 10 days is not significantly different from 5/8 in the control series. This is therefore to be regarded as a border-line result - i.e. the animals have not been immunised or made tolerant.

*Ho. 6. with very slight prolongation.*

PTO.

Histological report on the inoculated material taken from  
57.3, 5, and 6 at time of biopsy.

The injected material shows breakdown of very long standing. Nearly all fragments have organised fibrous capsules of native origin (surviving fibroblasts).

Inoculum from 57.2 (= 57.2.in). Total breakdown (but test graft 75% survival contemporarily). Note some fibrous capsule & surviving lymphocytes.

REPEATED INOCULATION OF NEWBORNS (A to cba) WITH EMBRYO  
TISSUE BREI THROUGH 3 ROUTES.

EMB-58

12 NOV 1952 DONOR TISSUE. 8 17/18-day fetuses representing litter 3A3/3 removed from 3A3/P female b. 26 April. Place at once into excess of warm Ringer. Use one for immediate extraction as below. Store three further in Petri dish at 5 deg.C on Ringer-soaked filter-paper. Store four others by lowering into cotton-stoppered 5/8ths inch test tubes without surrounding fluid and slowly freezing to -79 deg.C. in deep-freeze box.

Chop up embryo for immediate extraction coarsely and rinse with Ringer to get rid of excess red cells. Continue chopping to form a concentrated fine brei passing into No.26 BWG needle. This proved impossible, and a wider bore needle was used, about 2/3rd of the total embryo being aspirated into the syringe. The material injected consisted of a pretty dense suspension of cell clumps in a thin menstruum - not viscous, like the brei used in 57.

RECIPIENTS: cba litter from mouse of litter 2C2/2 b. 1 Aug. The litter of 6 was born during course of morning & were about 8 hrs old at first injection.

For injection, hold needle vertically in clamp, to get densest possible inoculum. Inject subcutaneously on both sides (0.01 ml each) and 0.015 ml intraperitoneally. In spite of large size of needle, there was little loss from the subcutaneous sites, but almost always some leakage from the i/p site. Two of the six found dead the following morning.

Store the residue if the injection matter in the Agla syringe at 4 deg. C for 24 hrs.

13 NOV 1952 (22 hr) Two mice had disappeared by this a.m., and one other was moribund. The remaining three were injected on the two subcutaneous sites only, with 0.015 ml of the suspension stored in the syringe at 5 deg. C overnight. These remaining three were pink and lively, but small; they will survive if not molested by the mother.

14 NOV 1952 (2) Only two now left, and these are somewhat undersized.

16 NOV 1952

The two survivors were not injected on the 15th because of their grossly backward condition. Re-inject each with 0.01 cc, subcutaneously, on each side of chest, and intraperitoneally with 0.02 cc. Operation successful. No. 26 BwG needle was used again as the high mortality rate after the first series of injections is thought to be due to the use of a coarser needle.

DONOR: 2 17/18 day old fetuses from 3A3/P, stored since 12 Nov. at -5. To facilitate chopping up the heads, limbs and vertebral column were removed. A very concentrated suspension was obtained.

18 NOV 1952

Reinject with exactly the same brei as used on 17 Nov. This had been stored in the syringe at -5 deg. Dose: 0.02 cc subcutaneously on each side of thorax, and 0.02 cc intraperitoneally.

Note: Whilst the brei contained a good many cells which seemed on the point of death, a high proportion had nevertheless remained viable.

21 NOV 1952 (9) Thaw out one of the deep-frozen embryos by plunging at once with constant stirring into 50 ml Ringer at 37 deg.C.

The embryo was dissected and a study made of the ease of preparation of its various parts by chopping them up individually with a scissors action of cataract knives. Skin was the most difficult, partly because bits larger than the needle bore simply refused to get sucked in, and partly because of the glutinous and self-clogging action of the bits. (After freezing anyway) liver, lung, kidney & heart were exceedingly easy to prepare, and bits much larger than the needle bore went up easily. These organs were, however, unavoidable slow-thawed (being internal), and this ease of preparation may be due partly to tissue damage. In general, however, preparation by cataract knives was easier and quicker than preparation by scissors where only small quantities were needed; further, the prepared material consisted of intact lumps of tissue of the maximum size for entry into the syringe.

In the end, a dense suspension of large lumps (virtually micrografts) of skin, liver, kidney, lung & heart were suspended in a relatively clear Ringer; concentration was effected by upending the syringe for half an hour before injection, followed by injection vertically downwards. The larger bore needle was used without any ill-effect.

Inject both babies with 0.2 ml in the two subcutaneous sites and 0.2 ml intraperitoneally.

25 NOV 1952 (13)

Thaw out one of the deep-frozen embryos as above and prepare a concentrated suspension from a small piece of skin, a piece of gut, liver, a kidney

and the heart. Inject each mouse subcutaneously on both sides of thorax with 0.02 cc and intraperitoneally with 0.02 cc.

Both young are now very healthy. Ops. satisfactory.

**Note:** A small skin graft was removed and transplanted to RTW of A-line female. This should be a fair test for the viability of the frozen embryos.

29 NOV 1952 (17) Thaw out the last but one of the stored (deep frozen) embryos by the usual quick method. Prepare what is essentially a fairly concentrated micrograft suspension (rather than brei) from the embryonic skin (individually chopped pieces), stomach, spleen, kidney, small amount of kidney, heart, gut. Inject 0.3 ml intraperitoneally and 0.3 ml subcutaneously in middorsal line into both mice.

4 DEC 1952 (22) Thaw out the last deep frozen embryo by the quick method. Prepare a very dense suspension of micrografts using the cataract knife technique of chopping up. Tissues used are: Skin, spleen, both kidneys, gut, heart and lung. The liver was rejected due to slightly messy appearance. Inject each mouse with 0.03 cc subcutaneously in mid-dorsal line, and 0.03 cc intraperitoneally.

**Note:** The foetal skin graft (deep frozen) transplanted to A-line mouse on the 25th appeared to have healed-in by the 7th day, but its appearance was very atypical - reddish and transparent, and altogether extremely delicate. It is possible that the area described in actual fact represents resurfaced granulation and that the graft has disappeared. To-day (9th day) the area is still pretty well uncontracted and square, more robust but still deepish red, and without signs of hair-growth.

9 DEC 1952

(27) Donor tissue: New-born litter (2 hours) from female of litter 4A1/1 pregnant by elder full brother. Has had cba homograft - see PREG-6. B. 13 Sept.

EMB-58

Use liver, spleen, kidneys, heart and a small piece of skin from two new-borns. Scissor method, wide bore needle, dense brei. The young mice were thoroughly washed with soap, distilled water and sterile Ringer before removal of organs. Store 5 new-borns at 5 deg. in usual way.

Inject each mouse with 0.03 cc i/p, and 0.04 cc subcutaneously in mid-dorsal line.

15 DEC 1952

(33) Donor tissue: From new-borns stored since 9 Dec. at 5 deg. Use two, discard rest. Tissues: liver, spleens, kidneys, hearts, lungs, and small piece of skin (the latter was separately cut up with catarract knives). Scissors method, dense suspension of largish particles, coarse needle. Inject 0.04cc subcutaneously in mid-dorsal line, and 0.05 cc i/p.

2 JAN 1953

(51) TEST OPERATION. DONOR: Cut & scrape two grafts from the close clipped and delicately dry-shaved skin of an A line female belonging to a litter born 10 Sept of mating 2A1/P female X 3A2/1 male. Transplant one each to 58.1 & 58.2.

58.1 Female. 16 g. Dorso-lateral approach. Skin with very thick fatty layer, carefully dissected away.

58.2 Male. 18 g. D/L approach. Operation O.K.

9 JAN 1953 (7)

1. Healing satisfactory, pull off cuticle to reveal graft roof with a raw and bleeding central area. Rims whitish in appearance but epithelial survival rather doubtful.

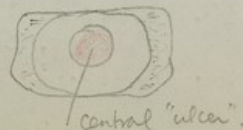
This seems to indicate that that the animal had been immunised, but even so the state of the graft must be considered as somewhat atypical. Also see notes on 2.

2. Healing faultless, cuticle off with tulle. Graft roof epithelialised and healthy pink, but graft is thinnish and delicate and with a slight haemorrhagic pattern. Survival probably considerable, and no strongly marked immunity effect can be observed.

[12 JAN 1953 (1) — Female. Notwithstanding 7-day appearance, graft has apparently settled down well and has a fully epithelialized surface. Graft is a little swollen..slightly rounded and domed...and surface is definitely delicate, though not sign of frank breakdown anywhere yet. Replaster.

(2) — Male. Much as (1) in general appearance except that it is worse on all counts. Graft very slightly domed; cuticle in form a rather thick plaques which, on removal, revealed a delicate but apparently fully epithelialized surface. Some signs of vascular disturbance in dermis. The centre of the graft was weak, and a circle of epidermis was in fact peeled off, to produce pseudo-ulcer in biopsy specimen. Some uneven discoloration on drying.

Specimen EMB-58, 2. Formol-HgCl<sub>2</sub>.





14 JAN 1953 (12)

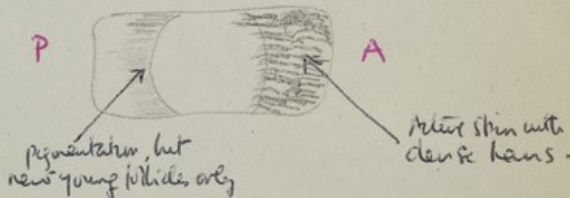
EMB-58

1. Graft uncontracted, healthy pink, flat, and with a dry roof. Cuticle very thin and delicate. No inflammatory symptoms. Do not rebandage, but merely apply vaseline.

15 JAN 1953 (13)

1. Graft more or less as above, still rather pink and delicate with possibly with one or two points of weakness.

17 JAN 1953 (15) AUTOPSY. Over the past 3-4 days the graft has been contracting, & it is now an oval  $4 \times 4\frac{1}{2}$  mm. The surface is very white, and rather scaly. Under the lens, the pattern of follicle nests can be easily made out, but there is no evidence of hair growth. The general appearance is a little too good to be consistent with overgrowth (and persistent dermal pattern of follicles tells against it); but very far from good enough for a normal graft. The whole history suggests a modified but not completely subdued reaction.



Specimen EMB-62:1. Specimen as shown in diagram. Formol-HgCl<sub>2</sub>.

Histological report.

58.1. Breakdown complete - extensive undermining. (15)

The graft dermis has almost completely disappeared and had been replaced by native fibres and resurfaced by native epithelium. This finding is in accord with the 7-day inspection report.

2. 75% survival. (10)

Breakdown complete over small off-centre strip, but rest of graft is predominantly alive. A strong reaction is building up - round cell population heavy but on the whole viable, local haemorrhages, tendency towards vacuolation and acidophilia of epithelial cells.

INOCULATION OF NEWBORN MICE (aaUU to A) WITH EMBRYONIC  
CELL SUSPENSION .

EMB-86

26 MAR 1953

Donor tissue: Remove the 14 days old embryos from female of litter 3B3/2, b.13 Nov., o-parous, pregnant by litter-mate. Chop up with scissors one whole embryo + gut, liver, spleen from another. Suck as much as possible through No.26 needle (about half of the raw-material), spin down and resuspend in small volume of Ringer. Suspension of cells and largish clumps thus produced rather glutinous.

Recipients: 3-5 hours old litter (9 in all) of female from litter 4A1/2, pregnant by litter-mate (= 5A2/P), b.10 Oct. This litter = 5A2/4.

Inject each young mouse with 0.03 cc of the suspension subcutaneously on each side, and with 0.02 cc intraperitoneally. Total dose 0.08 cc. When injecting subcutaneously the needle was inserted near the flank region and its point pushed into the area of the axillae.

All injections were satisfactory except for one which caused a haematoma.

30 MAR 1953

One mouse had disappeared. The other 8 are doing well.

19 MAY 1953 Test-operation.

Donor: Kill male of litter 3B3/1 M x 4B3/1 F, b.3 Feb., remove standard body-skin pinch grafts, trim off panniculus, and transplant one graft to each mouse.

Recipients: Graft bed prepared in usual way by scissors method. RHS ops.

1. F (R) 20 g.
2. F (L) 21 g.
3. F (no) 20 g.
4. F (both) 21 g.
5. F (2R) 19 g.
6. F (2L) 19 g.
7. M (R) 24 g.
8. M (L) 25 g.

20 MAY 1953 (10 = MST + 1). Breakdown very far advanced (or more probably complete) in all grafts. Epidermis peels with cuticle to expose white, damp surface with haemorrhagic blotches; graft browns off and scabs in air.

EXPERIMENT CONCLUDED

**28 MAY 1953**

Donor tissue: Kill male from A-line STOCK 1 (about 4 weeks old), remove spleen, half kidney, small piece of liver, and one testis. Chop up by standard method and prepare usual suspension of cells and clumps. Suspension thus obtained very dense.

Recipients: Litter of 8 from female ex2C2/6, b.5 March, pregnant by litter-mate or cousin, 1-parous, fed on special diet for some weeks. Each baby received 0.01 cc subcutaneously on each side of thorax, and 0.02 cc intraperitoneally --- total amount 0.04 cc. Injections satisfactory, but note that in a few of the intraperitoneally injections there was a certain amount of seeping back. The mother was rather upset by the removal of the litter but appeared to settle down in due course.

**1 JUN 1953** All 8 mice doing splendidly. ✓

**9 JUL 1953**

Test operation.

Donor: Male from litter 5A3/3 (b.8 Mar.) and female from same litter. Remove standard grafts and trim.

Recipients: 8 mice in all - do not number. Operations RHS and quite standard.

**16 JUL 1953** (7) Sample-inspection of 4 mice. Apart from well-defined pattern of vascular dilatation, the epithelium is firm & dermal pattern clearly marked: cuticle separates cleanly: no immune effect whatsoever.

20 JUL 1953 (11) Number mice this day.

1. M (R) Graft in excellent shape. Supermouse? Germolene.
2. M (L) " " " " " "
3. M (no) " " " " " "
4. M (both) Much as for 1, but slight dorsal fault which could be due to a specific inflammation; general appearance not quite as good as for 1. Germolene.
5. F (2R) 100% survival, but there is a ventral patch of inflammation, and general appearance not quite as healthy as that of 1. Germolene.
6. M (2L) Large central scab (on exposure), but rim of marginal survival. Advanced specific reaction.
7. M (no) Patchy survival, but extensive haemorrhagic patches and partial scabbing on exposure. Advanced specific reaction.
8. (2L) High degree of survival, but graft does not give too healthy an impression - slight discolouration in patches.

21 JUL 1953 (12)

1. Graft fully surviving, but surface rather shiny and there is the faintest discolouration near margin. Possibly slight contracture. Not very supple
2. Graft fully surviving, but patch (posterior) of discolouration. Not very supple.
3. Graft in very good condition, but small patch of the faintest discolouration.
4. Graft with dorsal scab, and whilst it is surviving it does not look too healthy - shiny and with faint discolouration. Not very supple.

5. Graft surviving, but ventral rim is inflamed and graft is generally slightly discoloured. Not very supple.
6. Scabbing complete. Kill. No prolongation.
7. Scabbing almost complete. Kill. No prolongation.
8. Much scabbing and contracture. Only a largish patch of survival.

### 22 JUL 1953 (13)

1. Graft fully surviving, but surface taut and shiny; no hairs; possibly some contracture.
- 8 8. Only a small patch of surviving epithelium remains - the rest of the graft has scabbed.
3. Graft O.K., but slight central discolouartion. Hairs are just beginning to penetrate anteriorly.
4. Garft much as yesterday - shiny, no hairs, not too hopeful.
5. Graft surviving, surface taut and shiny; no hairs; probably some contracture.
- 2 2. Graft O.K., slight posterior discolouration but incipient hair-growth.

### 23 JUL 1953 (14)

1. Little change. A few hairs have just erupted.
2. Little change - hair-growth more pronounced.
3. Graft surviving, but surface is shiny and with a patch of discolouration. Small anterior patch of hair-growth.
4. Little change - slight discolouration.
5. Little change.
8. Scabbing very nearly complete. Kill. Survival score 14 days.

**25 JUL 1953** (16)

1. Graft O.k. - hair-growth slow but developing. **Supermouse.**
2. Graft O.K. - good hair-growth. **Supermouse.**
3. Graft O.K. - hair-growth in progress. **Supermouse.**
4. Graft surviving, dorsal scab has almost 'healed', but surface glistening and bald; further contracture. **Not very 'super'.**
5. Graft O.K. but roof shiny and bald. Incipient hair-growth just perceptible dorsally. **Supermouse.**

**27 JUL 1953** (18)

1. Sparse hair-growth; pretty supple.
2. Bushy fur, ~~90 deg.~~ rotation, supple.
3. Sparse hair-growth, supple.
4. Little change; graft bald and not very supple.
5. Good hair-growth dorsally, but ventrally still bald and shiny; not very supple.

**29 JUL 1953** (20)

All grafts are doing well, but hair-growth definitely retarded compared with 106. Note that 4 may well be recovering; there has been some further contracture, but the dorsal scab has almost disappeared and a few white hairs are now visible.

**1 AUG 1953** (23) All grafts except 4 are O.K. 4 appears to have contracted further; the remaining part does not look too unhealthy but is bearing only a few hairs - still rather shiny.

**Photograph no.3.**

~~25 JUL 1953~~



6 AUG 1953(28)

The order of merit is: 2 - 5 - 3 - 1 - 4.

1. Graft round, definitely smaller than at first inspection, just palpable, bald (but matt, not shiny surface), rather white, and just a little bit too scurfy. Unquestionably 100% survival, but not a normal graft.
2. Graft excellent; hair growth not quite up to 106 standard; a little white.
3. Much as Graft 1 but a little better on all scores. There are wispy sparse hairs, and tiny little scabs on posterior ventral rim.
4. Much contracture; graft is dense and palpable, shiny white, bald.
5. Grafts has rather sparse hairs; but uncontracted. Dermal pattern is well defined; graft as a whole rather staring white.

14 AUG 1953 Litter of 5+ from the one female of the group. Destroy.

15 AUG 1953 (37).

1. Graft very white in colour and bears a sparse crop of short, wispy white hairs. There is central raised, discoloured blister centrally on which epithelium is weak & can be peeled. Elsewhere the epithelium is O.K. 100% survival.

2. Graft is perfect with excellent pelt of white hairs, not very far short of EMB-106 standard.
3. Ventro-posterior 1/3 of graft is heavily scabbed, elsewhere the graft is white in colour and bears ~~er~~ a sparse crop of wispy white hairs. ? is scabbing indicative of specific reaction.
4. Graft is very much contracted, ~~white~~ white, bald and generally scar-like. Probably no survival, though old graft dermis may form the fibrous matrix of the scar.
5. Graft white & uncontracted with prominent dermal pattern. There is a reasonable hair pelt.

Order of Merit unchanged i.e. 2 - 5 - 3 = 1 - 4.

**24 AUG 1953** (46)

1. Slight contracure has taken place; there is a remnant of a 'healed' scar dorsally. Graft is fairly supple and bearing a very sparse and patchy hair crop - it is clearly surviving but with all signs of a mild specific reaction which has been evident all the time but has been somewhat intensified since the beginning of this month.
2. Graft in good condition - thick pelt of fur.
3. Graft on way out? Scurfy anterior scab healing? There has been some contracture; graft is bald except for a few white hairs arising from the scab; rest of epithelium does not look too healthy. Clearly a specific reaction which began round about the 6th August.
4. 'Graft' is clearly a scar area. Breakdown has therefore been completed 6-15th August. Survival score: about 32 days. Keep animal for 2nd test graft to check for immunity. Note that this graft has shown signs of a mild and long drawn-out reaction from the very first; it has never grown hairs and its

general appearance throughout has been consistent with a mild specific reaction.

5. Graft in good condition, though pelt of fur is less dense than that of 2.

### 31 AUG 1953 (53)

1. Graft still in place and continuing to resist a mild and chronic reaction. It is small and rather shiny, but it has a patch of rather wispy white hairs. There is also a certain amount of orange discoloration. Graft clearly surviving.
2. Graft in very good shape. Supple; thick fur.
3. This graft is now inferior to 1. Its general appearance is similar but there are no hairs, and there has clearly been considerable contracture.
5. Graft in good shape. Supple; fur less dense than that of 2.

### 10 SEP 1953 ((63))

1. Graft only slightly larger than 3, but with a sparse crop of straggly white hairs.
2. Graft in fine shape.
3. Graft remnant now very small - about  $3\frac{1}{2}$  by 2 mm; but it is epithelialized and bearing a few white hairs which put its authenticity as graft tissue beyond doubt.
5. **Animal pregnant.** Graft in good shape if a little on the small side.
4. **Reoperate this mouse, this time on the LHS, to test for immunity.**  
Donor: Male A-line Stock 5 mouse.

**16 SEP 1953** (69)

1. Graft very small, delicately epithelialised - smooth and shiny and nearly completely bald. A few white hairs -straggly and somewhat disorganised- are however present and prove that some of the original graft epithelium survives. Nevertheless the chronic reaction which has been observed for some considerable time is clearly continuing.
2. Graft in good shape.
3. Graft area now exceedingly small and resembles scar tissue. Survival of graft epithelium is most unlikely. Survival time about 63 days.
5. Graft continues to be in good shape.
4. (6day inspection) Graft has healed well; cuticle comes off cleanly; graft robustly epithelialised - 100% survival. Slight reddening anteriorly. Rebandage.

**18 SEP 1953** (71) 4. (8 day inspection) This graft looks in fine shape and shows no trace of a reaction. Rebandage.

**21 SEP 1953** (74) 4. (11 days) Graft completely epithelialised and clearly viable, but somewhat delicate anteriorly. Thick cuticle comes off very cleanly.

**22 SEP 1953** (75) 4. (12 days) Graft now beginning to look leathery and a reaction is well under way. Still some survival.

**23 SEP 1953** (76) 4. (13) Graft looking very leathery - probably low degree of survival.

25 SEP 1953 (78)

1. Graft remnant bald, shiny, but delicately epithelialised. Probably still some survival, but another graft should help to elucidate the question of the ~~graft's~~ host's reactivity.
2. Graft in good condition.
3. This mouse should be regrafted.
4. Graft has now scabbed - survival time therefore about 14 days.
5. Graft in good condition.

15 OCT 1953 (88) REOPERATE EMB-108.1 and 3, this time on the LHS.  
 Donor: male from 7A1/3.

11 SEP 1953 (88 + 6).

1. Graft looks delicate & is pink but 100% surviving epithelium, & cuticle separated cleanly. Clearly functional vascular system. There was one tiny marginal blister which scabbed. Replaster.
3. Adherent cuticle, most difficult to pick off. Some survival certain; but general reddening. Replaster.
4. Destroy
- (5, 2: both grafts perfect).

13 OCT 1953 (88+8)

3. Removal of plaster reveals graft with very high degree of survival - slight central weakness. Don't rebandage.

**14 OCT 1953** (88+9) 3. There has been some contracture but the graft is showing a very high degree of survival.

**15 OCT 1953** 3.(88+10) Further contracture has taken place, but the graft is still predominantly surviving though ~~that~~ the epithelium is delicate and weak.

**16. OCT 1953** (88+11) 3. More contracture, some discolouration, but survival still reasonable. Note that the ~~course~~ course of breakdown is of the slow and lingering variety.

1. Graft fully epithelialised and looks in good shape.

Do not rebandage.

**17 OCT 1953** (88+12) 3. Graft still hanging on, but contracture is continuing. Breakdown is likely to be completed in a day or two.

1. This graft does not look too happy to-day - there has been some antero-dorsal contracture, the surface has the characteristic shiny appearance and is with one or two patches of discolouration. Note this appearance is completely in keeping with the behaviour of the first graft - i.e. there is a mild but sustained reaction.

**20 OCT 1953** (88+15) 3. Breakdown is now complete - survival time of this graft is therefore 14-15 days. Note that this is greater than the normal expectation of a homograft.

1. This graft has gone through a very sticky phase with all its usual symptoms: baldness, shininess, contracture, and

some superficial scabbing. The scabbing is mainly in the dorsal half. **BUT** white hairs are now beginning to grow in the ventral half.

**9 NOV 1953** (88 + 35) Graft on 108.1 is now reduced to an irregular much contracted outline with a few wispy white hairs growing from within it...aa **perfect borderline case of survival: graft just hanging on.**

Graft on 108.2 continues in excellent condition, as hitherto.

SKIN GRAFTING TEST

[Cf. also EMB-115 this day.] Under ether, <sup>of 108.2.</sup> cut standard pinch graft from rather thick but inactive skin of L thigh. Trim and transplant in usual way to **CBA Stock VIII female, no ear mark.**

**14 NOV 1953** (88 + 40)

1. Breakdown of this graft (the second) is now just about complete - Steady contracture, and the loss of white hairs, have made it almost impossible to be certain of any graft survival.

Score of first graft: 78 +  
" " second? " : 40

This animal has been a perfect example of a borderline case, all the way through. Another graft might in fact yield some further information.

2. Graft in good shape.
5. Mouse has had healthy litter which is still being suckled. The graft is clearly there, but hair-growth is thin and wispy.

**20 NOV 1953** Inspect the cba recipient carrying graft from lo8.2. (11 days)  
Cuticle is very strongly adherent and impossible to remove. Graft roof looks  
O.K. but may well be on the delicate side.

**21 NOV 1953** (12) Graft is gradually scabbing.

**22 NOV 1953** (13) Scabbing almost complete, but a small dorsal patch of surviving  
epithelium remains. Note that the graft is considerably depressed, i.e. the  
surrounding host skin is welling up above it. This is probably due to the fact that  
the host skin is intensely active.

**23 NOV 1953** The small dorsal patch appears to be surviving since it is  
distinguishable from the surrounding host skin. But it is impossible to be cer-  
tain that it is in fact graft epithelium. Eventually hair orientation  
may throw some light on this point.

**26 NOV 1953** (17 - Report on Skin Grafting Test of 9 Nov, continued). General  
contracture is evident. A dorsal segment (perhaps  $\frac{1}{3}$ rd) of the graft has  
a leaden-blue epithelium, are new agouti hairs, downwardly directed, are  
growing from it. Thus there seems no doubt about the partial survival.  
The ventral  $\frac{2}{3}$ ds of the graft is in fact a scab - not the usual yellowy  
cardboardy scab associated with the slow dying-out of a graft struggling  
to survive, but a frank traumatic scab which, when picked at, reveals a  
granulating and bleeding tissue beneath.

**28 NOV 1953** (19 - Skin graft test cont.) Graft remnant quite active and growing  
light brown hairs which are in contrast with the darker hairs of the host.



Report on 108 "supermice"

2. Graft in good shape.
5. Graft clearly in place, and there are white hairs growing in various parts of it; especially ventrally & there is a long wisp of hairs. But hair density and length is generally very poor - this graft has never settled down to a completely autograft-like existence.

30 NOV 1953(144)

(B) IMPLANTATION OF 108.2 WITH NORMAL CBA (ISOGENIC) NODE TISSUE

(A) SECOND INTRA-STRAIN SKIN GRAFTING TEST FROM 108.2 (cf. 115 also)

(A) Cut rather irregular graft from RHS thigh of 108.2 (normal inactive skin). Transplant to:- CX1/P male (normal bed).

Note:- The CX1/P pair derives from the 7th litter ex 2C2/6.  
The recipient is therefore an uncle of 108.2, and a full sib of its mother.

(B) Take both inguinal & all 4 axillary nodes from normal CBA mouse (Stock IX F). Chop into 33 bits. Individual nodes were small & flabby and milked only very slightly on cutting.

Implant these bits and the fluid they were suspended in into

mouse 108.2 intraperitoneally. Clean, effective insertion.

The resident A-line graft on 108.2 is in perfect condition.

[Purpose of this exp: to see if 108.2's nodes can be implanted with 'non-sensitized' node tissue from the same line, with the possibility that the resident A-line graft will provoke active immunity. Note however that these implanted node fragments cannot possibly enter the nodes draining the A-line graft.]

**1 DEC 1953** (88 + 56) Third stage graft on 108.1. Donor:- A-line Stock VIII male, active skin.

Transplant to RHS dorso-laterally (i.e. above old scar). The panniculus unfortunately punctured. Eosin plaster.

**3 DEC 1953** (146) Second stage graft on 108.5. Donor:- A-line VIII M. Normal skin. **LEFT HAND SIDE** operation.

[Note: The original RHS graft unchanged from last report: most of hairs are short and widely spaced except for long ventral tuft; but graft, though small, looks otherwise normal.]

[Graft on 108.2 (implanted normal nodes) looks perfect.]

3 DEC 1953

Final note on the <sup>hair</sup> cba recipient of skin  
from 108.2.

EMB-108

What must be presumed to be graft remnant (see previous notes) is growing a fair pelt of hairs at approx. 90 deg. to host hairs. Note that this graft has gone through a most severe reaction and that only a very small part of it has survived.  
KILL THE RECIPIENT.

4 DEC 1953

Second inoculation of 108.2 with normal CBA node tissue.  
(See Nov 30).

Excise 4 axillary, 2 inguinal, 2 cervical nodes (all small, flabby, 'non milky') from 6C7/P female. Trim & chop in usual way, and inoculate i/p into 108.2. Some bleeding (probably cutaneous).

[Graft on 108.2 continues perfect.]

5 DEC 1953

Graft on 108.2 shows no signs of a reaction - now 5 days after first implantation of nodes.

7 DEC 1953

Graft on 108.2 (now 7 days post-implant.) is still O.K.

6day inspection of 3dr stage graft on 108.1:

Healing is O.k. The whole of the graft, with the exception of a narrow annulus along most of the margin, is a raw, wet, and freshly bleeding area without epithelial survival. The surrounding annulus looks healthy enough, but it might well be host epithelium resurfacing an annulus of granulation.

Could there have been infection (since the graft is not mummified in usual way)? Severe immune effect is more likely.

79 DEC 1953

108.2. (9) Graft still in good condition.

Dimensions: A/P 8 mm  
D/V 6 mm

11 DEC 1953 11-day inspection of second test of Intra-strain grafts from 108.2.

Recipient: CX1/P male. Graft is completely autograft-like.

12 DEC 1953

108.2 (12 days after implantation of non-immune nodes):  
Graft in perfect condition - no trace of reaction.

14 DEC 1953 108.2 (14 days after implanting non-immune nodes). A/P dimension 8 mm: no contracture, & graft continues perfect.

108.5 (11 days after 2nd-stage grafting: 157 days after original test grafting). The 'new' (LHS) graft looked at first rather delicate; in fact epithelium was firm. ?Some contracture, however, after exposure to air. The RHS graft looked caked & generally scruffy, but the epithelium was in fact perfectly firm.

14-day inspection of second test of Intra-strain grafts from 108.2.

Recipient: CX1/P male: No longer perfectly autograft-like. A little reduced in area, & shiny and rather scurfy. But weak pigment shadow anteriorly suggesting onset of hair growth soon.

15 DEC 1953 108.2.: Graft still perfect.

108.5.: The new LHS graft has undergone considerable contracture ~~and~~ especially in the A/P plane, and it looks slightly discoloured; but graft clearly surviving. Host skin around it is intensely active.

16 DEC 1953

108.2 (144 + 16). Graft still perfect

108.5 (147 + 13). LHS (new graft): graft contracted AP & epithelium shiny & rather tissue papery; dorsal weakness under platelet scab.

RHS (old graft): contracted; swollen & palpable; & dark pink in colour.

Both grafts are definitely going through a specific reaction.

~~17 DEC 1953~~ 108.2 (144 + 17) Graft perfect.

108.5 (147 + 17) Both grafts are going through a very sticky period - contracture, some scabbing, and grafts are hard and palpable. B.d. is to be expected.

18 DEC 1953 (144 + 18) 108.2 graft dimensions  $7\frac{1}{2}$  mm A/P, 6 mm D/V. There is a tiny scab antero-dorsally & a very faint & possibly non-specific patch of reddening in the centre.

108.5 RHS still fair survival, bu scabbing posteriorly; LHS dorsal scab but good ventral survival.

20 DEC 1953 (144 + 20). 108.2 now  $6\frac{1}{2}$  A/P &  $5\frac{1}{2}$  D/V; scurfiness somewhat increased.

22 DEC 1953 (144 + 22) 108.2 shows further deterioration.

(147 + 19) 108.5 LHS graft: ventral half is surviving; RHS graft, though still a patch with white hairs, is in fact very hard and apparently on verge of complete scabbing. There could be central survival.

23 DEC 1953(23) Final inspection of second test on intra-strain graft from 108.2.

CX1/P M:- Graft has recovered; little contracture & the vigorous hair growth predicted at 14 days.

[FOR GENERAL SUMMARY, see under EMB-D5 this day]

24 DEC 1953 (144 + 24) 108.2 shows still further deterioration: area  $5\frac{1}{2}$  A/P x  $4\frac{1}{2}$  D/V; general scurfiness & scaliness & bunching up still further in evidence; graft now palpable.

108.5 There is now no clear evidence of survival in either graft; the areas surviving two days ago are now generally scabbed. Thus in this imperfectly tolerant supermouse, the application of a 2nd-stage graft has caused the breakdown of both. Interestingly enough, the 1st-stage graft suffered more quickly.

**26 DEC 1953**(144 + 26) 5 A/P x  $4\frac{1}{2}$  D/V. The graft is now harder, and a more extensive scab has formed anteriorly & ventrally. On lifting this off, a raw patch was exposed; but in spite of this the surface is still mainly epithelialized, and the epidermis in the D/P corner is still firm. Breakdown therefore well under way but not yet complete.

**28 DEC 1953**(144 + 28). (108.2 cont.) Total breakdown. The full thickness of the graft is now a dry scab, withered and mummified. Only a tiny D/P tongue of surviving epithelium remains, & this from its position & shape clearly represents ingrowth. SURVIVAL TIME 27 DAYS; first pathological changes seen at 18 days.

For second-stage grafting in due course.

**13 JAN 1954**

(144 + 42) SECOND STAGE GRAFT ON 108.2. Donor: A VIII male. LHS opn.

(186) THIRD STAGE GRAFT ON 108.5. Donor: v.s.; mid-dorsal opn. This female is in late pregnancy.

**19 JAN 1954**

108.2. 6-day inspection of 2nd stage graft: violent immune effect - macerated surface without epithelial survival.

This result again points to the continued existence of the implanted ~~imm~~ non-immune node cells in the host's body - note that the tempo of the immune effect is perfectly typical and not like

that of a ~~normal~~ 2nd stage graft on an ordinary tolerant mouse whose first graft had broken down.

108.5. 6-day inspection of 3rd stage graft: typical very strong immune effect - free rim, mummification, weak surface, no epithelial survival.

Test-operation on ex-108.5 (F) x 108.2 (M) litter, b.5 Nov.

Donor: Stock 8 A-line male. Standard grafts and normal RHS operations. 5 recipients. Use as MST as primary hosts for PT-37.

30 JAN 1954(11) All grafts show typical MST picture. Use as blood donors for PT-37 q.v.



20 AUG 1952 Donor tissue: aaUU cells (exactly as EMB-25 q.v.).

RECIPIENTS: The first litter of mouse X-2 (b.30 May = 82 days old, pregnant by cba litter mate; notch L ear and clip mark anteriorly).

6 young, almost certainly not born this morning (no milk in stomach) and injected 3.30 p.m., hence maximum  $5\frac{1}{2}$  hr old. Each was injected with 3 x 0.01 ml dense adult cell & cell-clump suspension in the following places: R shoulder region & L shoulder region subcutaneously; intraperitoneally. Then inject each with 0.05 mg cortisone subcutaneously in dorsal midline region.

23 AUG 1952 (3) Litter injected with 0.05 mg cortisone - all O.K.

26 AUG 1952 (6) Reinjecting with 0.05 mg. cortisone - all O.K.

2 SEP 1952 (13) All 6 O.K.

8 SEP 1952 (19) One baby dead. 5 remain, but 2 are particularly retarded and have little chance of survival.

11 SEP 1952 One more dead, as expected.

21 OCT 1952 Test Operation.

DONOR: 2BI/2 male, 29 g. Operation as for EMB-25.

I.S. F. (R) 18 g. Fit fair. Small nick in skin prior to ops. included in ant. part of bed, but graft not resting upon it.

2. F. (L) 18 g. Close fit.
3. M. (no) 22 g. Close fit.
4. M. (both) 16 g. Close fit.

29 OCT 1952 (8)

1. Healing faultless. Cuticle off with tulle, Graft roof dampish and weak, very white with a few haemorrhagic patches. Survival probably rather low.  
Rebandage.
2. Healing faultless. Cuticle off with tulle. Graft very large. Roof fairly dry but with extensive haemorrhage. Small blister near centre.  
Rebandage.
3. Healing faultless. Cuticle off with tulle. Roof wet and eroded as well as haemorrhagic. Little survival.  
Rebandage.
4. Healing faultless. Cuticle tending to adhere. Largish graft. Roof haemorrhagic but fairly dry. Some survival possible.  
Rebandage.

3 NOV 1952 (12)

1. Pull off thick and scabby cuticle. Roof very wet and white. Some pastiness. Survival unlikely.  
Specimen EMB-28-I. Cut out strip with host skin at one end only.  
Formol/Hg.
2. Roof very white, wet and pasty. Obviously no epithelial survival. No biopsy.
3. Lift off scabby cuticle - roof moist and white. On exposure ventral half becomes deep red - this part is obviously de-epithelialised.

Dorsally there may some little survival.

EMB-28

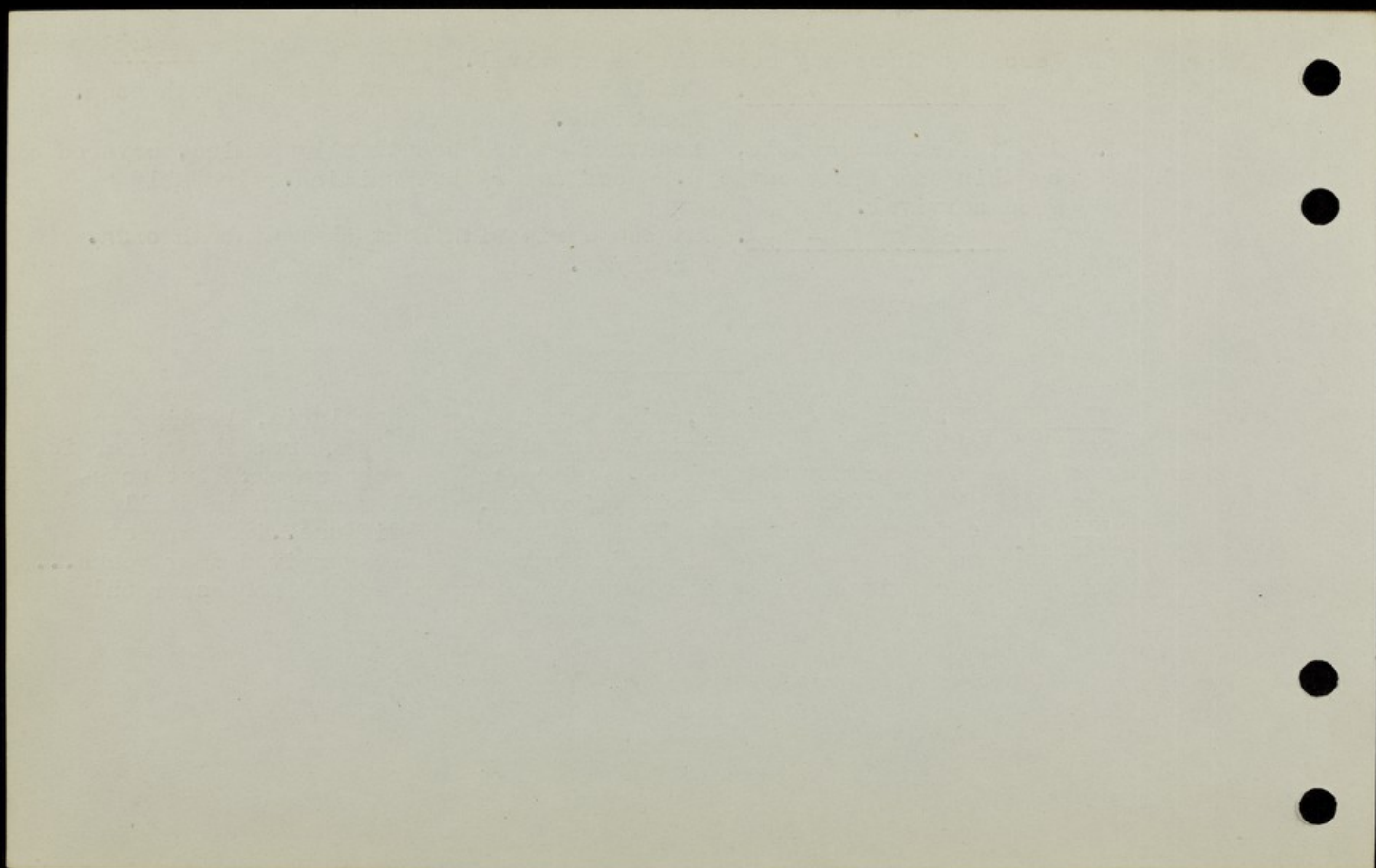
Specimen EMB-28-3. Cut out strip with host skin at both ends.  
Formol/Hg.

4. Graft flat and supple, uncontracted and beautifully healed, covered by thin and flaky cuticle. Roof is dry but whitish. Probably good survival.

Specimen EMB-28-4. Cut out strip with host skin at both ends.  
Formol/Hg.

#### HISTOLOGY

SPECIMEN 28.4 100% survival, with persistent hyperplasia, leading to complete obliteration of original dermal collagen pattern: the epidermis now presents a pretty well plane surface to the substratum. But note good differentiation of new hair primordia. Cell reaction is still 'live' (no fragmentation of cells) but weak and diffuse..indeed, not strong enough to suggest that graft would not have survived many weeks... except under bridge epithelium between graft and normal host surround.



**31 JUL 1952** Donor tissue: 3A3/1.6 female 22 days old. Remove both kidneys (with adrenals attached), the spleen, and a piece of liver about the same size as the spleen. Chop up under Ringer with scissors to get a sense suspension of single cells, together with a number of small 'organized' clumps. These latter were heavy, and not many probably went in. Large nos. of cell nevertheless certainly did.

Recipients: litter 2C4/2.1-6, b.6 pm approx 30 July & hence 16 (sixteen) hrs old approx. at injection. Each member of the litter was given a subcutaneous injection of 0.01 ml in the left shoulder region; ditto in the R shoulder region; ditto intraperitoneally. In later injections, the micrometer & syringe were held, not horizontally, but at an angle of about 20° only off the vertical, to persuade the heavier cell clumps to go through.

**2 AUG 1952** (2) All O.K.

**6 AUG 1952** (6) Litter O.K., but mother dead. Litter therefore given to foster-mother with 3 young of her own of about the same age.

Note that foster-mother is an A-line animal.

**7 AUG 1952** (7) Litter doing well.

**10 AUG 1952** (10) Litter in splendid condition.

**25 AUG 1952** All 6 very healthy.

**2 SEP 1952** Segregated.

26 SEP 1952 TEST OPERATION.

DONOR: A-line female (2A6/I), born 18 May, 26 g. Remove and trim grafts as for EMB-I8, and transplant using exactly the same method.

1. F. (R), 19 g. Fairly close fit. Small puncture dorsally.
2. F. (L), 20 g. Close fit.
3. F. (no), 19 g. Fairly close fit.
4. M. (both), 23 g. Fairly close fit.
5. M. (2R), 21 g. Fairly close fit.
6. M. (2L), 25 g. Fairly close fit.

4 OCT 1952 (8)

1. Healing faultless. Graft looks thin, and its surface is pink with very fine punctate appearance. General impression is of a fairly healthy graft.  
Rebandage.
2. Healing faultless. Apart from one or two small points of redness (which could easily be non-specific) graft seems in excellent shape,  
Rebandage.
3. Healing faultless. Cuticle rather adherent- pull off. Surface dry and pink and healthy in appearance.  
Rebandage.
4. Healing faultless. Cuticle off with tulle. Same as for 2.  
Rebandage.
5. Healing faultless. Graft swollen, haemorrhagic, sponge-like, and lacking suppleness. Specific reaction probably far advanced.  
Rebandage.

EMB-I7

6. Healing faultless. Cuticle off with tulle. Graft dry and healthy pink but with faint signs of incipient haemorrhage.  
Rebandage.

**EB OCT 1952** (12)

1. Thick cuticle, peel off. Graft very thin. Surface slightly moist, and rather white with redness showing through. There appears to be some surviving epithelium.  
Specimen EMB-I7-1. Strip with host skin at each end. Formol/Hg.
2. Dry and very thin cuticle. Graft looks in good shape, flat, supple, dry and delicately pink. Autograft-like.  
Specimen EMB-I7-2. Strip with host skin at each end. Formol/Hg.
3. Scaly cuticle off to reveal surface which to be epithelialised marginally but central area with severe inflammatory symptoms.  
Specimen EMB-I7-3. Strip with host skin at each end. Formol/Hg.
4. Graft appears to be autograft-like in all respects - healthy colour, strong roof. But cuticle remains adherent. Probably very high degree of survival.  
Specimen EMB-I7-4. Strip with host skin at each end. Formol/Hg.
5. Graft completely necrotic - advanced disintegration.  
No biopsy.
6. Peel off thick damp cuticle. Surface glistening and weak, its colour being deep pink and centrally reddish. Marginal annulus seems fairly healthy, but central area one of healed ulceration on exposure to air. Breakdown probably far advanced.  
Specimen EMB-I7-6. Strip with host skin at each end. Formol/Hg.

### Histology.

1. 5% survival.  
Slight trace.
2. 75% survival.  
Limited epithelial breakdown is taking place, but the graft is predominantly viable. Vascularisation is sound, and many capillaries are intact. There is quite a fair round cell population in the graft bed, but the round cells both here and in the graft are on the whole viable. General impression one of a modified reaction.
3. 5% survival.  
Follicular trace.
4. 100 survival. POTENTIAL SUPER-MOUSE.  
No tangible indications of imminent breakdown, though some cells are somewhat vacuolated and slightly pyknotic. Considerable round cell infiltration into the graft dermis, but cells on the whole viable. Some have penetrated into the epidermis itself. New follicles are being laid down, but so far little sebaceous gland tissue. Vascularisation good, and many vessels are intact.
6. TBLS.  
Little remains of the graft dermis -this is reminiscent of immune breakdown. Cf. notes on 5.

Conclusion: 2 grafts out of 6 show a very significant prolongation. Compare this with other New-born expmts.

Note that these animals were fostered by an A-line female from the 6th day.



INOCULATION OF NEWBORN HYBRID MICE WITH LEUCOCYTE  
CONCENTRATE (aaUU to F<sub>1</sub>)

EMB-152

**18 DEC 1953** DONORS:- Withdraw 3 ml whole blood from two B-line Stock II females. At once mix in with 10 ml of 1% dextran solution (M.W. 400-500,000) in normal citrate saline. Allow to stand for 15'; then spin gently. Decant supernatant and respin firmly. Take up sediment in 0.5 ml dextran-saline. Red cells were present in about the concentration of normal blood; allowing for loss, it can be taken that the leucocytes were present in at least X4 the normal conc. (At most X6).

RECIPIENTS:- 8/11 progeny representing a second or third litter derived from the hybrid mating A-line Stock V female X CBA Stock V male. These were born sometime in the afternoon and were injected at 6.30 p.m. i.e. were 3-4 hours old. Inject each mouse with 0.05 ml concentrate intraperitoneally; in the majority there was a loss that might amount to 0.02 ml.

**4 FEB 1954** Test-operation.

Donor: B-line Stock 2 female. Standard grafts. RHS operations.  
7 mice. (uo)

**15 FEB 1954** (11) 5/7 - advanced b.d. but slight survival.  
1/7 - b.d. complete  
1/7 - complete survival, 100%, no ominous signs.

16 FEB 1954 (12) 6 grafts: b.d. complete.  
1 " : high degree of survival, but slight scabbing  
has begun. Prognosis poor.

17 FEB 1954 (13) Only survivor: Advanced scabbing, but about 25% survival.

18 FEB 1954 (14) Breakdown now complete. Score: 14 days. i.e. slight  
prolongation.

Note absence of an immune effect in this series.

REPEATED MULTIPLE INJECTION OF NEWBORN MICE WITH  
CONCENTRATED EMBRYONIC TISSUE BREI (cba to A).

EMB-62

**25 NOV 1952** DONOR TISSUE. From cba female of litter 302/1 (b.28 Aug) remove the three 14-day embryos (pregnant within line). Use one as below; store other two (unnecessarily removed from amnion & uterus) in usual way at 5 deg C. Also remove & store spleen, 2 kidneys & piece of liver; use as under EMB-58 q.v.

Chop up the entire 14-day embryo with scissors to form a somewhat gelatinous suspension containing large lumps that slid into small-bore (26) needle. Nearly the whole of the embryo in brei or minced form was injected into the 7 recipients.

RECIPIENTS: A-line newborns of the litter 4A1/3 (see stockbook). 8 were born between 11 a.m. and 4 p.m., and injected at 6 p.m., hence **age 7 hours at the extreme**; more probably about 4 hrs, since babes were clean & afterbirth cleared up. Only 7 survived to injection.

Injections as follows: 0.005 ml subcutaneously in axilla; 0.01 ml in middorsal line subcutaneously; 0.01 ml intraperitoneally.

**27 NOV 1952** (2) Donor tissue: Whole embryo from 3C2/1 stored since the 25th at -5 deg. Brei similar to that previously used.

**Reinject** each mouse (there are only 4 left) as follows:

0.03 cc subcutaneously in mid-dorsal line.

0.04 cc intraperitoneally.

0.02 cc subcutaneously on left side of thorax.

Note: The mid-dorsal injection is not to be recommended at so early a stage - it causes too large a bleb and loss of fluid is greater.

29 NOV 1952 (4) Brei prepared from whole embryo stored at 5 deg.C since 25 NOV; scissors method, all tissues used. 4 youngsters remain; inject 0.01 subcutaneously on both sides; 0.02 intraperitoneally; 0.02 subcutaneously in middorsal line. Brei was concentrated; injections successful. Fine bore needle.

1 DEC 1952 (6) New injection material. Kill C-line pregnant female of litter 2C3/4 (b.31 Aug); excise the 5 circa 15-day fetuses in utero and store four as such at 5 deg C on damped filter paper. Using cataract knives chop up (a) about 5 mm<sup>2</sup> skin and (b) the viscera; aspirate into No. 26 needle to form a dense micrograft (rather than ~~the~~ brei) suspension, and inject 0.5 ml intraperitoneally and 0.3 ml into each side subcutaneously. The 4 youngsters are doing exceedingly well; in future use wider-bore needle.

3 DEC 1952 (8) Use tissues from stored fetus of 2C3/4. Prepare dense suspension of largish micrografts from heart, both kidneys, piece of liver, spleen, and small piece of skin. Use cataract knives to chop up. Inject litter as follows: 0.02 cc intraperitoneally, and 0.01 cc subcutaneously in mid-dorsal line. This may seem a small dose, but due to tendency of micrografts to settle out the supernatant was rejected and the remaining suspension exceedingly concentrated. Coarse needle used. In future a more uniform brei should be obtainable by using scissors for chopping up. The whole embryo can be used with a coarse needle provided that the skeleton is first removed.

5 DEC 1952 (10) DONOR: 2 circa 14-day foetuses from 2C2/3 female, b. 2 Sept, pregnant within line. Scissor method, dense brei, wider bore needle, 0.04 ml i/p, 0.02 subcutaneously on each side.

Store remaining 6 foetuses at 5 deg. C. in usual way, discarding the earlier batch of embryos. Strage in utero.

9 DEC 1952 (14) Donor tissue: 3 foetuses from 2C2/3 stored at 5 deg. since 5 Dec. Scissor method, dense brei, wider bore needle. 0.03 cc. i/p, 0.03 cc subcutaneously on each side.  
Note: Age of foetuses probably nearer 13 days.

13 DEC 1952 (18) Donor tissue: Kill 3C1/P female, b.15 June, and remove the 18-day approx foetuses representing litter 3C1/4. From two of these, remove & chop with scissors: skin, liver, 2 spleens, 4 kidneys, 4 lungs, part of gut, 2 hearts. Suspension consisted of large lumps in thin fluid, rather than a brei; use wide-bore needle.

Inject 0.04 ml subcutaneously in dorsal midline; 0.03 ml i/p.

Store the remaining embryos at 5 deg.C.

18 DEC 1952 (23) Donor tissue: Remove lungs, hearts, kidneys, spleens, a little liver and some gut from 3 foetuses (3C1/4) stored at 5 deg. since 13 Dec. Scissors method, very dense brei, coarse needle. Inject each mouse with 0.04 cc subcutaneously in mid-dorsal line, and 0.04 cc intraperitoneally.

**22 DEC 1952** (27) This litter has now been segregated. Donor tissue: remove 7 1 $\frac{1}{2}$  day fetuses from CBA female of litter 2C2/3 (3 Sept), pregnant within line. Store 3 in utero on Ringer-filter paper at 5 deg.C. Chop up remainder with scissors and make a very concentrated micrograft + brei suspension, representing perhaps two whole embryos.

Inject 0.06 ml subcutaneously in mid-dorsal line far forward and 0.06 ml intraperitoneally.

**27 DEC 1952** (32) Donor tissue: make a brei + micrograft suspension, v. concentrated, of the three embryos left in store since 22 DEC. With allowance for loss, about 2 whole embryos injected into the 4 animals thus:- 0.04 ml on each side subcutaneously, and 0.07 ml intraperitoneally.

**2 JAN 1953** (38) There are 3 males & 1 female, the former large & vigorous, the latter very much undersized. Look into at autopsy.

**13 JAN 1953** (49) TEST OPERATIONS on 62:1,2,3 (males); not on 62:4 (female: grossly undersized, & to be examined on a later occasion).

Donor:- 3 body skin grafts from male of litter 3C5/2, b.11 Nov.

Recipients:-

62.1 M. R ear. LHS op. 21 g.

62.2 M. L ear. LHS op. 22 g.

62.3 M. No mrk. RHS op. 22 g. This mouse had a palpable

nodule on each side of thorax just above site normally used for transplantation. Investigate at autopsy.

20 JAN 1953 (7)

14.

1. Healing faultless. Cuticle off with tulle. Graft roof wet and severely haemorrhagic. Breakdown almost certainly complete: clear-cut immunity effect.  
Rebandage. Biopsy at median survival-time (10).
2. Healing faultless. Cuticle adherent- pull off some to reveal severely haemorrhagic but as yet pretty dry graft roof. Some patches are clearly still epithelialised, but even here the surface looks weakish and the reaction is probably well under way: weak immunity effect.  
Rebandage. Biopsy at m.s.t.
3. Healing faultless. Cuticle off with tulle, but some hairs adhere obstinately. Graft roof dry and epithelialised, without sign of an inflammatory reaction: no immune effect.  
Rebandage. Inspect at m.s.t.

23 JAN 1953 (10-M.S.T.)

1. Only a very eroded graft dermis remains; breakdown is quite obviously complete and of very long standing. Hence no biopsy. Search for remains of the inoculum: again a few small strands subcutaneously in region of thorax, and the now familiar nodules, chiefly loose and without doubt non-viable, in the body-cavity. Remove these and fix in Formol/Hg. Specimen 62-1 (In).

2. Appearance of graft leaves no doubt that breakdown is complete, though erosion of graft dermis does not compare with 1 in this series. **No biopsy.**  
Remains of inoculum: again a few subcutaneous strands and several white pellets lying loose in body-cavity. Fix in Formol/Hg.  
Specimen 62-2 (In).
3. Graft autograft-like. Flat, uncontracted, greyish (pigmented), and undoubtedly epithelialised. Flaky and rather waxy cuticle. No bandage - vaseline only.  
**Correlated with the subcutaneous nodules noted at time of ops. this is an interesting result.**

**26 JAN 1953** (13)

3. Whilst about 2/3 of the graft is pretty well autograft-like (except for slight swelling), a scar has developed ventrally and is almost certainly due to a specific reaction. It began to appear yesterday and has since become enlarged and scabby. Elsewhere the graft is grey (pigmented) and follicle-formation appears to be taking place.

**28 JAN 1953** (15)

The scar noted during the last few days is being shed and and the graft seems to be healing in this region. A number of hairs are beginning to sprout around the scar, but most of them



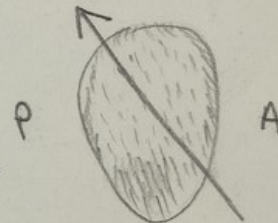
are whitish. The rest of the graft is clearly pigmented and epithelialised, but the general impression is one of turgidity, and there is as yet no active hair-growth. The surface has a completely un-autograftlike shine, and it is clear that the graft is suffering from a mild reaction which is likely to be cyclic in character.

6.

30 JAN 1953 (17)

3. Graft no longer swollen or turgid-looking; deep leaden blue in colour throughout; **agouti hairs** about 1-2 mm long ventrally (original scar position) as shown.

Clearly visible hairs also around lateral and dorsal margin. Bino inspection shows evenly distributed black hairs over rest of graft. Hair orientation as indicated by arrow.



Max.AP  $4\frac{1}{2}$ , DV 7.

Test Operation. (of runt).

Donor: 4B3/1 male, b. 6 Oct.

4. Female. 10 g. Ops O.K.

Note: This animal was inadvertently test-operated with a B-line graft. Therefore wait until breakdown of this graft and then replace with cba graft.

Small nodule representing homologous tissue found just below panniculus of graft bed. Remove carefully and fix in Formol/Hg.

~~2~~ FEB  
JAN 1953

No. 4 (runt) has died over the week-end. It has almost certainly been dead for more than 24 hours and a post-mortem was not revealing.

3 FEB 1953

(21) Graft in excellent shape and is now almost completely covered by a dense coat of bushy agouti hairs. Hair-growth extremely active.

10 FEB 1953

(28) Photograph graft, first with its natural fur and then with hairs clipped. Fur very dense and bushy. On clipping the graft was revealed to be well pigmented, with clearly defined graft margins, and still shield-shaped. Dimensions: Max. AP 5mm, DV 9mm. This if anything represents an increase in area. Graft roof healthy, but near the left dorso-ventral margin there is a slight ridge of particularly active hair-growth, and also a slight suspicion of remnant of a scar. This is where the original scaring noted on the 26 Jan. had occurred.

Graft in excellent shape.

17 FEB 1953

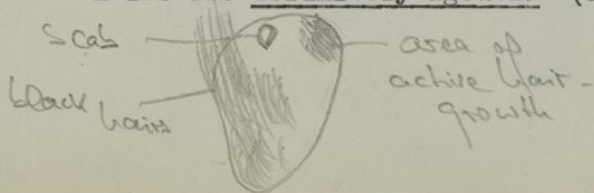
(35) Graft definitely in place and uncontracted. It is growing dark hairs, but whilst these are long and bushy along a line more or less following the anterior margin (see diagram) the rest of graft is covered rather thinly.

Note: P.B.M. has noted the very black pigmentation of the graft hairs which is entirely anomalous. L.B. remembers that the hairs before clipping also seemed unusually dark.

2 MAR 1953 (48) Graft O.K. Hair-growth seems to be a little <sup>7.</sup> more active now but coat of hairs is still rather patchy - chiefly anteriorly and dorsally. The large subcutaneous cyst noted at time of test-operation is still present.

Unfortunately this animal has developed, during the last fortnight, a skin 'rash' on the back of the neck. At first it was thought that scratching may have been responsible, but the affected area is now too extensive and the scabbing too severe for this explanation to hold water. D. Mickey suggests that Ectromelia is unlikely as scabs are too bloody and on an unusual part of the body. It is possible that there has been infection by mites, thus causing severe scratching. Disinfect.

5 MAR 1953 (51) Graft O.K. except for a very small scab near the dorsal margin. This is probably non-specific as far as a homograft-reaction is concerned, and may well be caused by scratching (see above). There has been no contraction - dimensions: 8mm by 5 mm. Margins of graft still clearly visible. Patches of hairs, but large part remain bald or very sparsely covered. Dry-shave: Dorsally, quite near the margin, is a small area with active hair-growth. Here the epithelium is deeply pigmented and the hairs are definitely agouti. (in contrast to those elsewhere - black).



The scabs on other parts of the body of this mouse are responding to treatment - there has been a great improvement.

6 MAR 1953 (52) Graft O.K. Scar has not increased in size and is almost certainly due to scratching.

8 MAR 1953 (54) Graft is beginning to show signs of pigmentation which is gradually spreading across surface. Short agouti hairs are sprouting from around the remnant of the scar, which has healed almost completely.

17 MAR 1953 (63) Graft covered by a dense crop of agouti hairs.

23 MAR 1953 (69) It was planned to reoperate mouse and transplanting to it yet another cba graft. Prior to this opn. the primary graft was photographed with the animal under nembatal, and death occurred whilst this procedure was being carried out. Hence:

1. Transplant  $\frac{1}{2}$  of primary cba graft, together with some host A-line tissue, to A-line animal of litter 4A4/3, b.12 Feb., male. Ops. satisfactory, RHS, rather open fit. Cba part of graft situated dorsally, A-line part ventrally. Usual opn. procedure.
2. Transplant the other half of the primary cba graft, together with some albino skin at one end, to cba male of litter 4C2/2, b.6 Feb., RHS, slightly open fit, opn. satisfactory.

This experiment should show clearly that it is the host which has been altered by the initial inoculation, and not the grafted tissue.



4A4/3  
4C2/2

P.T.O.

Post-mortem on Emb-62.

Huge cyst located subcutaneously latero-dorsally on left side. This mass of tissue is composed of a large number of lumps, some pigmented and with hairs, which have fused together chiefly by the hosts fatty connective tissue - i.e. it is not a cyst in the strict sense of the term. All appearance of being well vascularised. Attachment to subcutaneous layers and also to some extent to the musculature.

Another very much smaller mass subcutaneously on right side, and yet another subcutaneously attached to body-wall in midline of abdomen. A somewhat larger mass attached to connective tissue of gut - several different types of tissue again & distinguishable, some being pigmented. Small cyst near spleen.

Small cyst (homogeneous and bladder-like) in lobe of liver.

Small cyst, with several components, attached to lobe of lung, but entirely external.

**Wet weight** of all this homologous tissue: 1.3 g.

Fix all in Formol/Hg. Specimen EMB-62.Inoc.

All organs are macroscopically normal except for cyst in lobe of liver.

**31 MAR 1953** (8)

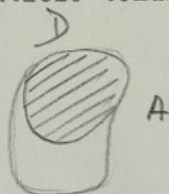
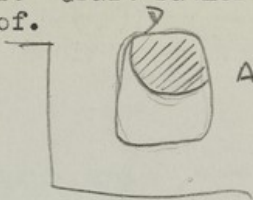
4A4/3: Healing of composite graft faultless.

White part: Cuticle off cleanly with tulle. Healthy, very light pink colouration. A few tiny points of non-specific haemorrhage .

Agouti part: Cuticle tends to be somewhat adherent - some hairs remain in position after pulling off. Graft rather haemorrhagic but as yet with dry roof.

4C2/2: Healing of composite graft faultless.  
Albino portion: Cuticle off cleanly with tulle. On the whole little sign of a reaction, but ventrally the roof is dampish and haemorrhagic.

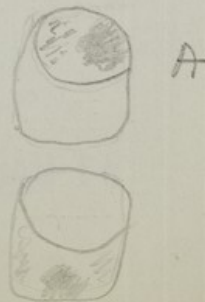
Agouti portion: Cuticle tends to adhere. On the whole quite autograft-like.



2 APR 1953 (10)

4A4/3: Albino component: Quite perfect - flat, supple, thin scaly cuticle, same colour as host skin.  
Agouti component: Does not yet show complete breakdown, but anterior part is ulcerated, rest rather brownish discoloured. Definitely some surviving epithelium. Germolene and rebandage.

4C2/2: Albino component: Already ulcerated ventrally, and epithelium peels away to give white glistening surface which turns brown. Total breakdown.  
Agouti component: Small fresh defect dorsally but surface is firm and a bit scaly. Slight pigment dilution.



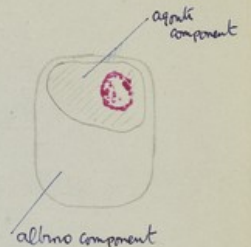
No evidence of breakdown, but probably some inflammation.

9.

4 APR 1953 (12)

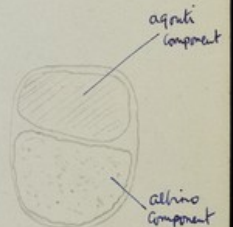
4A4/3. The albino component of the graft from EMB-62 is in every respect perfect.

The agouti component shows a fairly advanced reaction, but to outward appearance it does not seem quite complete; a slightly dusky colour remains and there is no contracture. On picking at the epidermal surface, the epidermis came away to reveal a naked but freshly-bleeding dermal surface (indicated in red in diagram). Thus the reaction, though in progress, is in some respects anomalous and must be regarded as delayed.



4C2/2. The albino component of the graft from EMB-62 has been reduced to a yellow dry scab which simply picked off to reveal granulation tissue below. Total breakdown of long standing.

The agouti component is better than in 4A4/3; outwardly, it looked a little raised and rather scurfy. Careful picking at the posterior margin revealed a brisk inflammatory reaction with vascular dilatation in the dermis below. There is therefore no doubt that this graft is eliciting some reaction, though a rather feeble one.



Germolene on both grafts; no plaster.

(PTO.

(Comments. Note that these results assume the complete interchangeability of skin within lines; this may not be always fully justified, but it is certain that early reactions cannot be due to residual heterozygosity within lines.

The albino component results are perfectly straightforward. At present it looks as if the agouti component is causing some reaction on the agouti recipient, instead of being completely acceptable, and is enjoying a prolonged survival on the albino recipient. In weighing up these results it should be borne in mind that much of the stroma (including new collagen) of the agouti graft on its albino host EMB-62 will be of albino origin.)

6 APR 1953 (14)

4A4/3. Albino part still perfect; CBA part a tiny withered scab. Breakdown very long complete.

4C2/2. Albino part long since withered away. CBA part is covered by a thick, rather crinkly epithelium, which remains firmly attached. Its edges show a trace of scabbing, representing the effects of the epithelium-stripping test done last time. Whole graft is somewhat raised, but more as if fibrotic than as with fresh oedematous swelling. Survival is unquestionable; note also that graft is very easily identifiable as such.



**10 APR 1953** (18)

10.

4A4/3. Albino part growing short white hairs. Kill animal.

4C2/2. Agouti part uncontracted and epithelialised but with clearly abnormal dermal pattern. No hair-growth. The graft has obviously not yet recovered from the mild inflammation which it has been subjected to. This reaction was presumably directed against host cells which had infiltrated into the graft dermis during hte graft's homologous residence. There can however be little doubt that the graft epithelium survives.

**14 APR 1953** (22)

4C2/2. Graft now well pigmented but still no hair-growth. Surface rather shiny.

**15 APR 1953** (23) 4C2/2. Graft is beginning to show hair-growth at one or two points - hairs certainly agouti.

**17 APR 1953** (24) Hair-growth at several points continuing. Orientation of hairs dorso-ventral, not antero-posterior as in surrounding skin. Graft still easily identifiable as much of it is still bare. The hairy points are not particularly marginal and overgrowth by host epithelium can be ruled out, especially since the graft has been watched daily and has undoubtedly remained intact inspite of the mild reaction noted earlier.

22 APR 1953 (30) Graft has grown a bushy crop of agouti hairs which are definitely at 90 degrees to the direction of the host's hair-growth. On clipping the outline of the graft is clearly visible. No doubt at all that this is a fully surviving and indeed flourishing graft.

Destroy animal.

Histological report.

EMB-62. Inoc.

Only half of the main cyst has been sectioned. It consists of a mass of many different tissues, all of which show a high degree of differentiation, as well as complete survival. The following tissues are clearly identifiable:

Liver lobules

Bone

Cartilage

Skin epithelium with abundant melanocytes.

The skin epithelium (present in large quantities) shows follicular activity - note also its encystment with central masses of exfoliated cuticle. Melanocytes are numerous and are present even in the mesenchymal tissue. All tissues are well vascularised; there is some limited round cell infiltration but all cells are viable.

INJECTION OF NEWBORN HYBRID MICE WITH ADULT WHOLE  
BLOOD (aaUU to F<sub>1</sub>).

EMB-151

**18 DEC 1953** DONOR:- As for EMB-150, except that the blood was withdrawn at 6 pm yesterday and has been stored at room temp overnight.

RECIPIENTS:- The 8 progeny representing the third litter derived from mating a Stock V CBA female with an A-line Stock V male. Inject each mouse with 0.05 ml whole blood i/p. Some slight leakage in all.

The litter had not been born at 6 p.m. last night: its maximum age is therefore 15 hrs; its probable age 8 hrs.

**6 JAN 1954**. One died at weaning age.

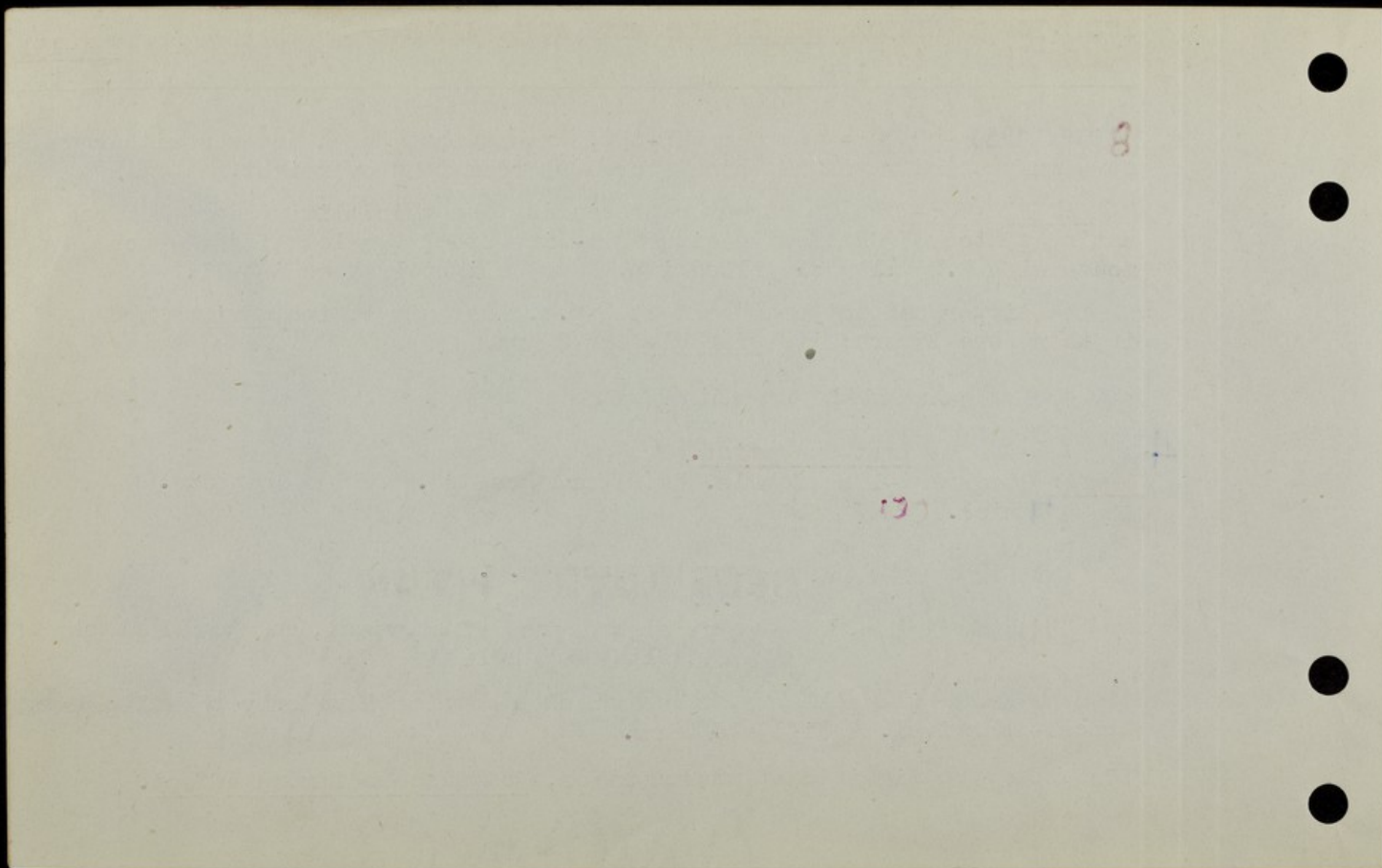
**4<sup>th</sup> FEB 1954** Test-operation.

Donor: Stock 2 B-line female. Standard grafts . RHS operations.  
7 mice. (2)

**15 FEB 1954** (11) 3/7 - very advanced b.d.  
3/7 - b.d. in progress, but fair marginal survival.  
1/7 - very high degree of survival, but small patch of peeling epithelium

**16 FEB 1954** (12) All grafts have broken down or have only the slightest trace - survival. **Kill all mice.**

**Note**: no significant prolongation, but also no immune effect.



8

19

INJECTION OF NEWBORN HYBRID MICE WITH ADULT WHOLE  
BLOOD (aaUU to F<sub>1</sub>).

EMB-150

**17 DEC 1953** DONOR:- Stock II B-line female. Recover 1.4 ml whole blood & mix with about 0.1 ml Na Citrate.

RECIPIENTS. The 5 surviving progeny representing the third litter derived from a mating of a CBA Stock V female with an A-line Stock V male.

Inject each mouse with 0.05 ml i/p. Some slight leakage in all cases.

Injections were done within 3 hours of birth.

**4 FEB 1954**, Test-operation.

Donor: Stock 2 B-line females. Standard grafts. RHS operations.  
5 mice. (L)

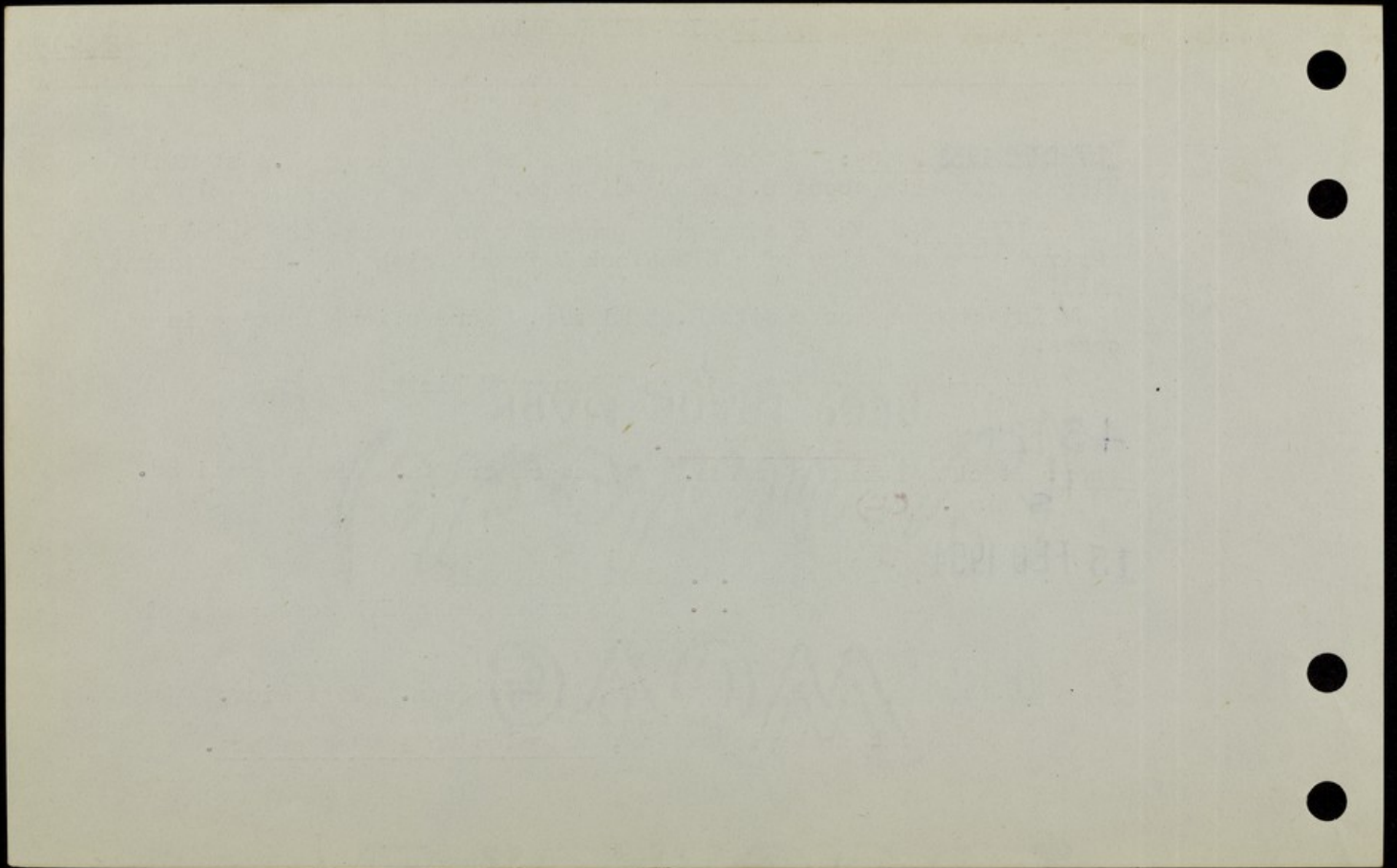
**15 FEB 1954** (11) 2/5 - b.d. almost complete  
3/5 - b.d. in progress but still some marginal survival.

**16 FEB 1954** (12) All grafts have broken down. Kill mice.

Note: no prolongation, but also no immune effect.

R 1954

FEB 1954



INJECTION OF NEWBORNS B (AU to A) WITH MIXED ADULT  
WHOLE BLOOD AND CELLS FROM SPLEEN & NODE

EMB-159

**16 JAN 1954 DONOR:** Female mouse of litter 8B2/1, N.B. First withdraw 0.6 ml blood by cardiac puncture, adding about 0.1 ml 4% citrate. Then excise inguinal nodes, 5 (NB) axillary nodes and two cervical nodes, and the spleen. Pass through medium bore hypodermic by usual 'syringe method', and from the expressed tissue, by pipetting, prepare a moderately dense suspension of expressed cells. Mix an equal volume of this suspension (also citrated) with the whole blood, and use for injection. Essentially the medium consists of 50% whole blood enriched with node & spleen cells.

**RECIPIENTS:-** 8/12 newborns of the litter 8A6/4.1-12. Birth first marked this a.m., probably overnight. Inject each i/p with 0.01 ml, noting loss of at least half in all cases.

**18 JAN 1954** The litter is being looked after very successfully.

**26 FEB 1954** Test-operation.

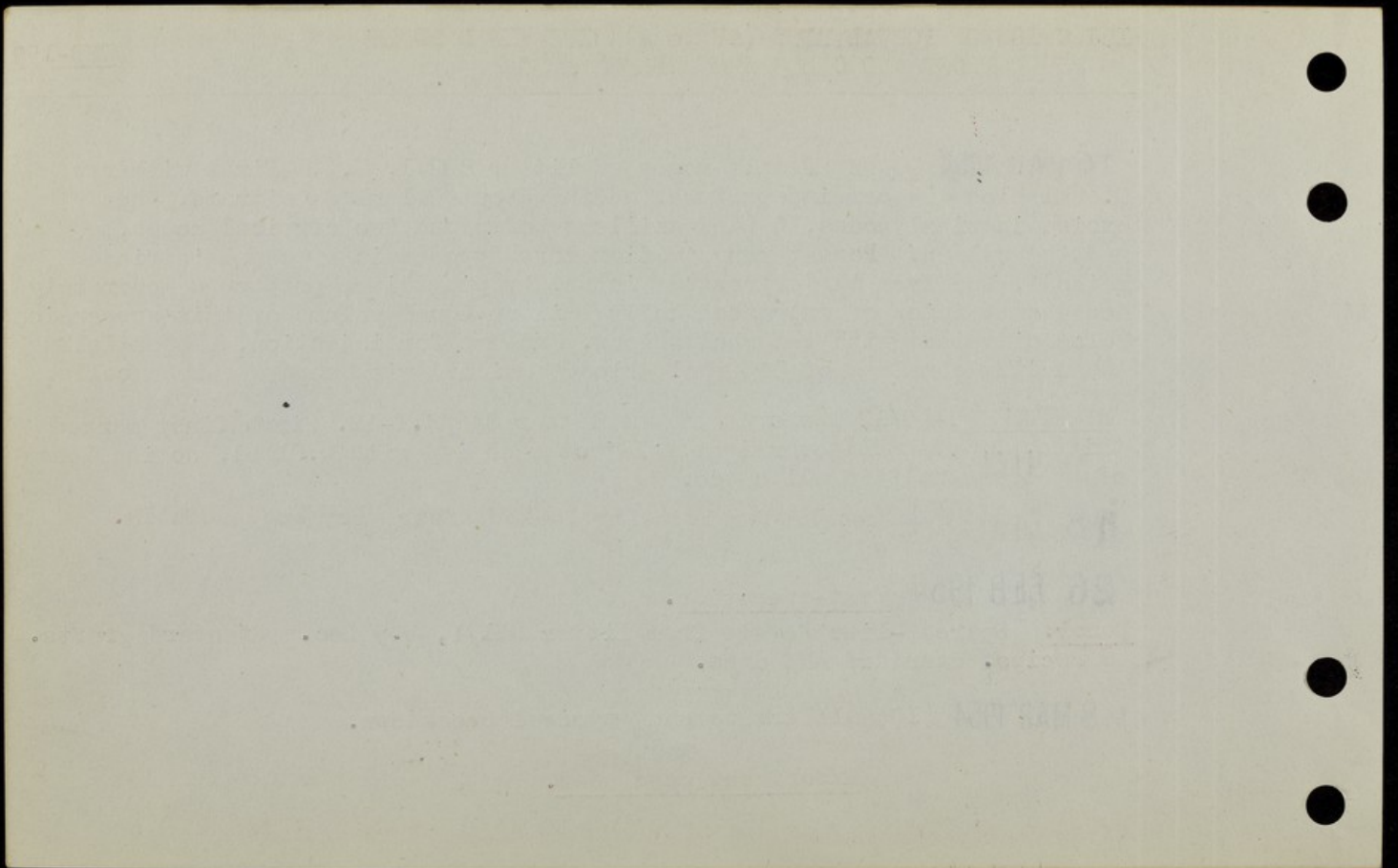
**Donor:** Spare B-line female from litter 8B1/1, b.5 Dec. Standard grafts. 8 recips. Standard RHS opns.

**8 MAR 1954** (10) All grafts show typical breakdown.

Experiment concluded.

2) 8 FEB 1954

SEP 1954





**DONOR TISSUE:** 6 aaUU embryos, 14½ days old, from line female of 2B1/1 litter (born 21 April), pregnant by litter mate or first cousin of 2B series. The donor tissue was **stored overnight** (see below).

**RECIPIENTS:** The new-born 2nd litter of 8 from 3A2/P (parents born 3 April 1952). Pedigree number of litter = 3A2/2.1-8.

These mice were born sometime between 7 pm July 14th and 9 am July 15th, and were injected between 10.15 and 10.30 am on July 15th. Hence **not more than 15 hrs old at extreme**, and probably about 8 hrs old.

Each baby was injected with a No.12 needle as with EMB-7 q.v.; the cell suspension was dense and included many organized tissue clumps. The quantity injected was not measured accurately, but generally exceeded 0.01 ml.

Classify litter as

8.1, 8.2 , .... 8.8.

**15 JUL 1952** Kill the pregnant aaUU female at about 5 pm 14 July and put embryos at once in warm normal saline. Then transfer to wool & cellophane stoppered centrifuge tube without adding further saline, and store at 5 deg.C. approx overnight. At 9.30 am 15 July, chop up in usual way with scissors and prepare a dense cell suspension, with many small organized lumps, in normal saline. The suspension contained bony matter which tended slightly to clog the 26 BWG syringe needle used in EMB-9, but was far easier to handle than the older suspension of EMB-7. A very satisfactory preparation. **For use see also EMB-9.**

1 SEP 1952 Test Operation.

DONOR: aaUU male, 2B6/2. Take standard body-skin grafts, remove panniculus by scraping and transplant to RTW clipped bed in usual way.

Note: Only the males of this litter were test-operated as the females were as yet under weight.

1. M. (no) 21 g. Close fit.
2. M. (R) 19 g. Close fit except posteriorly where there is a largish gap with punctured panniculus.
3. M. (L) 20 g. Fairly open fit.
4. M. (both) Close fit. Dorsal and ventral snicks.

9 SEP 1952 (8)

1. Only ventral rim of graft adherent to bed - rest completely mummified and general impression is one immune type of breakdown. Kill.
4. Healing very satisfactory. Cuticle off with tulle. Graft roof very moist, with orangy colouration and diffuse haemorrhagic patches. Slight puffiness. Very low degree of survival.  
Rebandage. Take biopsy at 10 days.
3. Healing quite satisfactory. Graft roof very damp and suspiciously white. Slight signs of a vascular reaction. Possibly some survival.  
Rebandage. Take biopsy at 10 days.
2. Healing satisfactory. Cuticle off with tulle to reveal wet roof with extensive haemorrhagic patches. Even a low degree of survival unlikely.  
Specimen EMB-8-2. Cut out dorso-ventral slot with host skin at each end.  
Formol-Hg.

1 1 SEP 1952 (10)

EMB-8

3. Graft roof white and wet. No cuticle. Obviously no epithelial survival. Tendency to "brown-off" on exposure.  
Specimen EMB-8-3. Cut out strip with host skin at one end. Formol/Hg.
4. Graft roof white and damp. No cuticle. Breakdown probably complete.  
Specimen EMB-8-4. Cut out strip with host skin at each end. Formol/Hg

1 6 SEP 1952 Test Operation of females.

Donor: aaUU male, from litter of 2B3/I, about 8 weeks old. Take standard body-skin grafts, remove panniculus by scraping and transplant to RTW clipped bed in usual way. Normal saline was used to wet scissors. The 4 females were operated in this way. Op. very satisfactory.

5. F.(no) 19 g. Close fit. Slight bleeding during ops.
6. F.(both) 21 g. Close fit.
7. F.(2R) 19 g. Somewhat open fit.
8. F.(2L) 19 g. Close fit.

2 3 SEP 1952 (8)

5. Healing satisfactory. Remove cuticle, Graft roof with conspicuous haemorrhagic patches, especially posteriorly. Some degree of survival likely. Rebandage.
6. Healing perfect but graft roof moist and with haemorrhagic patches. Rebandage.

7. Healing good. Graft surface fairly dry and white with two severe haemorrhagic patches dorsally. Slight puffiness. Rebandage.
8. Healing perfect, though graft accidentally torn from granulation along ventral margin. Graft roof distinctly haemorrhagic but dry. Rebandage.

25 SEP 1952 (10)

5. Graft severely discoloured dark brown to black, and altogether scabby in appearance. B.D. obviously complete.  
Specimen EMB-8-5. Cut out strip with host skin at each end. Formol/Hg.
6. Severe discolouration - brown scab with messiness in centre.  
Specimen EMB-8-6. Cut out strip with host skin at each end. Formol/Hg.
7. Exactly the same as for 6.  
Specimen EMB-8-7. Cut out strip with host skin at each end. Formol/Hg.
8. Graft roof very moist and with red haemorrhagic areas. B.D. likely to be complete.  
Specimen EMB-8-8.

Histology.

2. B.D. complete.
4. T.B.L.S.
8. T.B.L.S.

**DONOR:** 7 16-17 day embryos from aaUU strain F., born 21 APR 1952, primiparous. Female is of litter 2B1/1 & is pregnant either by litter mate or by first cousin of one of the litters of the 2B series.

**RECIPIENTS:** First litter of 6(+ 2), injected within 100 minutes of birth, of Strong-A female 2A1/2.5 (b.26 APR 1952), primiparous, pregnant by 2A1/2.6 male. Litter number 3B5/1.1-8.

**11 JUL 1952** Kill donor F by dislocating neck; remove the 7 embryos and put into warm normal saline; decapitate embryos and chop up with scissors in centrifuge tube, preparing tissue suspension in usual way.

A wider-gauge needle (No.12) was used for inoculating the embryos. As the donor tissue was from rather older embryos, the needle easily became clogged; but because of its wide bore, fairly large tissue fragments were included among the finer cell matter.

The 6 recipient embryos (8 in all; but 2 were cyanotic and later died) were injected subcutaneously by passing the needle forward from the sacrum up to the base of the neck in dorsal midline or just to one side of it. At first an attempt was made to inject a measured quantity by the micrometer syringe; but as the needle track left a tunnel from which a variable quantity of the inoculum escaped, this was abandoned for the injection of a quantity judged by the size of the bleb formed. Expression from needle a bit jerky, because some of the tissue clumps were large.

All embryos received at least some tissue suspension including the larger organized fragments.

11 JUL 1952 (18 hrs) The 6 newborns are being reared with both parents and seem OK.

1 SEP 1952 Test Operation.

DONOR: aaUU male, 2B6/2. Take standard body-skin grafts, remove panniculus by scraping and transplant to RTW clipped bed in usual way.

Note: Only the males of litter EMB-7 were test-operated to-day because the females were still under weight.

1. M. (no mark) 21 g. Rather open fit.
2. M. (R) 22 g. Fairly open fit.
3. M. (L) 21 g. Close fit. Dorsal and ventral snick in panniculus.
4. M. (both) 22 g. Slightly open fit.

9 SEP 1952 (80)

1. Graft almost 100% necrotic and appears to be a technical failure. No evidence of infection.
2. Healing unsatisfactory - graft is flat and in position but only ventral is united to bed and seems to be well vascularised. Cuticle adherent, pull off from ventral region. Surface very moist and dark orangy-pink in colour. Note that dorsal half lies on a perfectly good bed and has nevertheless failed to heal. **This does suggest that animal was already immune. Kill.**

Specimen EMB-7-2. Cut out dorso-ventral slot with host skin at one end. Specimen looks slightly ragged.

3. Healing satisfactory, cuticle off with tuje. Graft roof very moist and with diffuse haemorrhagic patches on a white background. Degree

of survival probably very low due to specific reaction.  
Rebandage. Take biopsy at 10 days.

4. Exactly as for 3. A perfect fit and healing very satisfactory but decidedly advanced phase of breakdown.  
Rebandage. Take biopsy at 10 days.

**11 SEP 1952 (10)**

3. No cuticle. Graft surface white and extremely white. A few brownish marks. Tendency to "brown off" on exposure. Obviously 100% B.D.  
Specimen EMB-7-3. Cut out strip with host skin at each end.  
Formol-Hg.

4. No cuticle. Graft roof fairly moist and with brownish colouration. Almost scabby in appearance. Whitish rim may be overgrowth.  
Specimen EMB-7-4. Cut out strip with host skin at each end.  
Formol/Hg.

Note: Specimen may have a little host epithelium along its length.

**16 SEP 1952** Test operation on females of litter.

Donor: aaUU male, from litter of 2B3/I, about 8 weeks old. Take standard body<sup>6</sup>skin grafts, remove panniculus by scraping and transplant to RTW clipped bed in usual way. Normal saline was used to wet scissors. The 2 females were operated in this way. The ops. was very satisfactory.

5. F.(R) 20 g. Close fit.
6. F.(L) 19 g. Close fit.

23 SEP 1952 (8)

5. Healing very good. Cuticle adherent but on removal exposes healthy-looking pink graft roof which is quite obviously epithelialised. Reddish weak patch at 5 o'clock at margin. Rebandage.
6. Healing satisfactory but roof is very moist and somewhat discoloured - degree of survival almost certainly very low. "Browning-off" on exposure to air. Rebandage.

25 SEP 1952 (10)

5. Graft dark brown, hard and scab-like, and somewhat raised. No doubt that B.D. is complete.  
Specimen EMB-7-5. Cut out slot with <sup>wo</sup> host skin at each end. Formol/Hg.
6. Central region brownish in colour. Marginally a white annulus of overgrowing native epithelium.  
Specimen EMB-7-6. Cut out slot with host skin at each end. Formol/Hg.

Histology.

- 7.2. 5% survival.  
Slight follicular trace.
- 7.3. Breakdown complete.
- 7.6. Breakdown complete.



**15 AUG 1952** DONOR TISSUE. Exactly as EMB-18 q.v., except that a fresh suspension was prepared from stored intact tissue lumps (see EMB-18). Storage 24 hrs at about 4 deg.C.

RECIPIENTS. Litter 2C1/3.1-7, age approx 8 hrs (cannot possibly be more than 14½ hrs).

Inject subcutaneously as for EMB-18 and intraperitoneally. All injections went off well.

After injection of cells, inject 0.05 mg/(0.002 ml standard Merck suspension), as with EMB-14, under skin of dorsal midline. Clearly defined white crystalline deposit.

**18 AUG 1952** All 7 O.K. Now reinject each with 0.05 mg cortisone.

**21 AUG 1952** Reinfect each with 0.05 mg. cortisone under dorsal midline. All 7 O.K. and very lively. The cortisone has had a less pronounced effect than has been observed previously and growth-rate cannot be very far from normal. Pigmentation is retarded, but not excessively so, whilst hair-growth is definitely backward.

**2 SEP 1952** All 7 O.K.

**7 OCT 1952** TEST OPERATION.

DONOR: A-line male, IAI/4. Remove 7 standard full-thickness body-skin

grafts from flank and side, trim by scraping off the panniculus, and store temporarily on Ringer-moistened filter-paper. Transplant one graft to each mouse (but not to the mother) on to a RTW scissor-clipped bed. Ringer used to wet scissors. Dressings as usual.

1. M. (R) 2I g. Open fit.
2. M. (L) 2I g. Fairly close fit.
3. M. (no) 2I g. Close fit.
4. M. (both) 23 g. Fairly open fit.
5. M. (2R) 2I g. Close fit.
6. F. (R) 19 g. Open fit.
7. F. (L) 18 g. Close fit. Dorsal puncture.

15 OCT 1952 (8)

1. Healing faultless. Cuticle off with tulle. Graft roof dry and healthy pink with fine red punctate pattern. Rebandage.
2. Healing faultless. Cuticle off with tulle. Graft roof dry and healthy pink. Rebandage.
3. Slight ventral undertuck - healing otherwise satisfactory. Cuticle off with tulle. Graft conspicuously inflamed and degree of survival probably low. Rebandaged.
4. Healing faultless. Cuticle adherent - pull off. Graft roof moist weak and generally with intense red patches indicative of an advanced specific reaction. Rebandage.

5. Exactly as for 4.
6. Healing faultless. Cuticle off with tulle. Roof dry and healthy pink.  
Rebandage.
7. Healing faultless. Cuticle off with tulle. Roof dry, well keratinised, mat, and healthy pink. Autograft-like.
8. Rebandaged.

19 OCT 1952(12).

1. Peel off dry cuticle to reveal pinkish coloured graft surface which is only very slightly damp. Impression is that there is still some surviving epithelium. There is a fairly large obviously unsurfaced area at 10 o'clock near margin-this became scab-like on drying.  
Specimen EMB-19-1 Strip with host skin at ends. Formol-Hg.
2. At first site graft looks perfect, being healthy pink colour. Peel off thin cuticle to reveal slightly moist surface which may have some surviving epithelium- only trace survival if any.  
Specimen EMB-19-2 Strip with host skin at ends. Formol-Hg.
3. On removal of cuticle roof appears moist. There is central haemorrhagic patch and entire graft darkened on exposure to air becoming scab-like. Survival highly improbable.  
Specimen EMB-19-3 Strip with host skin at ends. Formol/Hg.

4. Cuticle off with tulle. Graft looks like dead white, moist collagen pad. Almost certainly no survival.  
Emb.-19-4 Strip with host skin at ends. Formol-Hg.
5. Cuticle off with tulle, surface moist & graft haemorrhagic overall. Became scab-like on exposure to air. Survival highly improbable.  
Specimen EMB-19-5. Strip with host skin at ends. Formol-Hg.
6. Thick, damp cuticle off with tulle which may represent entire epithelium. Graft is moist and haemorrhagic and became scab-like on drying. Almost certainly no survival.  
Specimen EMB-19-6. Strip with host skin at ends. Formol-Hg.
7. Pick off thick cuticle to reveal slightly damp graft roof which is white in colour and almost certainly without surviving epithelium. Most unhopeful appearance on drying in air.  
Specimen EMB-19-7. Strip with host skin at ends. Formol/Hg.

#### Histological Report.

1. 10% survival.  
Survival is follicular and epidermal. Graft small and clearly contracted. Extensive overgrowth. Healing O.K. but poor vascularisation.
2. 5% survival.  
Difficult to distinguish between surviving epithelium and native overgrowth. The latter is certainly extensive, and breakdown elsewhere of some standing.
4. 10% survival.  
Pretty fair follicular survival, but cells very indolent. Vascularisation very slight.  
SINCE THE OTHER GRAFTS ALMOST CERTAINLY HAVE NO SURVIVAL? THIS LITTER DOES NOT DIFFER SIGNIFICANTLY FROM THE CONTROL GROUP.

Note: this is essentially a repeat of EMB-17.

14 AUG 1952 DONOR TISSUE. Kill 3A1/1.7 M, 36 days old, and aseptically remove spleen, both testes, both kidneys, and about  $\frac{1}{2}$ g liver. Cut up into large lumps and divide each into three parts. Store 2 parts of each on saline-damped filter paper in Petri dish at 4% C. for later use. Treat other one-third part as follows.

Chop fine with scissors in centrifuge tube, adding normal salt. Aspirate into micrometer syringe through No.26 needle: microscopical examination of actual injection fluid shows very dense suspension of single cells (rounded and epithelioid), small organized cell clumps (kidney epithelial clumps easily identifiable), and a good deal of articulate cell debris.

RECIPIENTS. The first litter of 4 (but see below) from cba female Y-1 (primiparous, b.12th June: see Survival Time Book). These were injected, holding the syringe only little off the vertical, in three places with 0.01 ml in each: left shoulder region subcutaneously, right shoulder region subcutaneously, intraperitoneally. **One of the four young killed** because of herniation of intestine. Some fairly clear fluid always emerged from intraperitoneal injection point, but undoubtedly some cells and cell clumps stayed in.

Age: the recipient babies had not been born at 6 p.m. 13 AUG & thus cannot be more than  $15\frac{1}{2}$  hrs old at the extreme. From general appearance particularly of mauled corpse, 6 hrs is probably as good a guess as any.

15 AUG 1952 *One further death, leaving two survivors.*

2 SEP 1952 Both O.K.

26 SEP 1952 TEST OPERATION.

DONOR: A-line female (2A6/I), born 18 May, 26g. Remove standard full thickness pinch grafts from flank and side, remove panniculus by scraping, and store temporarily on Ringer-moistened filter-paper. Transplant one to each mouse on to RTW scissor-clipped bed. Dressings as usual. Ringer used to ~~wet~~ scissors.

1. M. (R), 23 g. Fairly close fit.
2. M. (L), 23 g. Fairly close fit.

4 OCT 1952

(8)

1. Healing faultless. Cuticle fairly adherent - pull off. Roof dry and with a healthy pink colouration, but there are a number of fine points of redness which may be indicative of incipient haemorrhage.  
Rebandage.
2. Healing perfect. Graft in excellent shape and autograft-like in every respect.  
Rebandage.

8 OCT 1952

(12)

1. Graft looks healthy pink, flat, supple, and altogether in good condition. Ventrally there is some moistness which is probably indicative of a reaction. But high degree of survival.  
Specimen EMB-18-1. Strip with host skin at each end. Formol/Hg.
2. Peel off thick, moist cuticle which may include the entire epidermis. Before removal of cuticle graft surface looked weak but

not altogether unhealthy, but subsequently centre became very red and scab-like. Probably little survival, if any.  
Specimen EMB-I8-2. Strip with host skin at each end. Formol/Hg.

Histology.

2. T.B.L.S.

1. 5% survival.

A number of cells are only just viable and scattered in an otherwise dead epithelium. Active skin - thick cuticle and cystically dilated but dead follicles.

Note absence of immune effect.

