HYB [hybrids] 1-21 [+ index]

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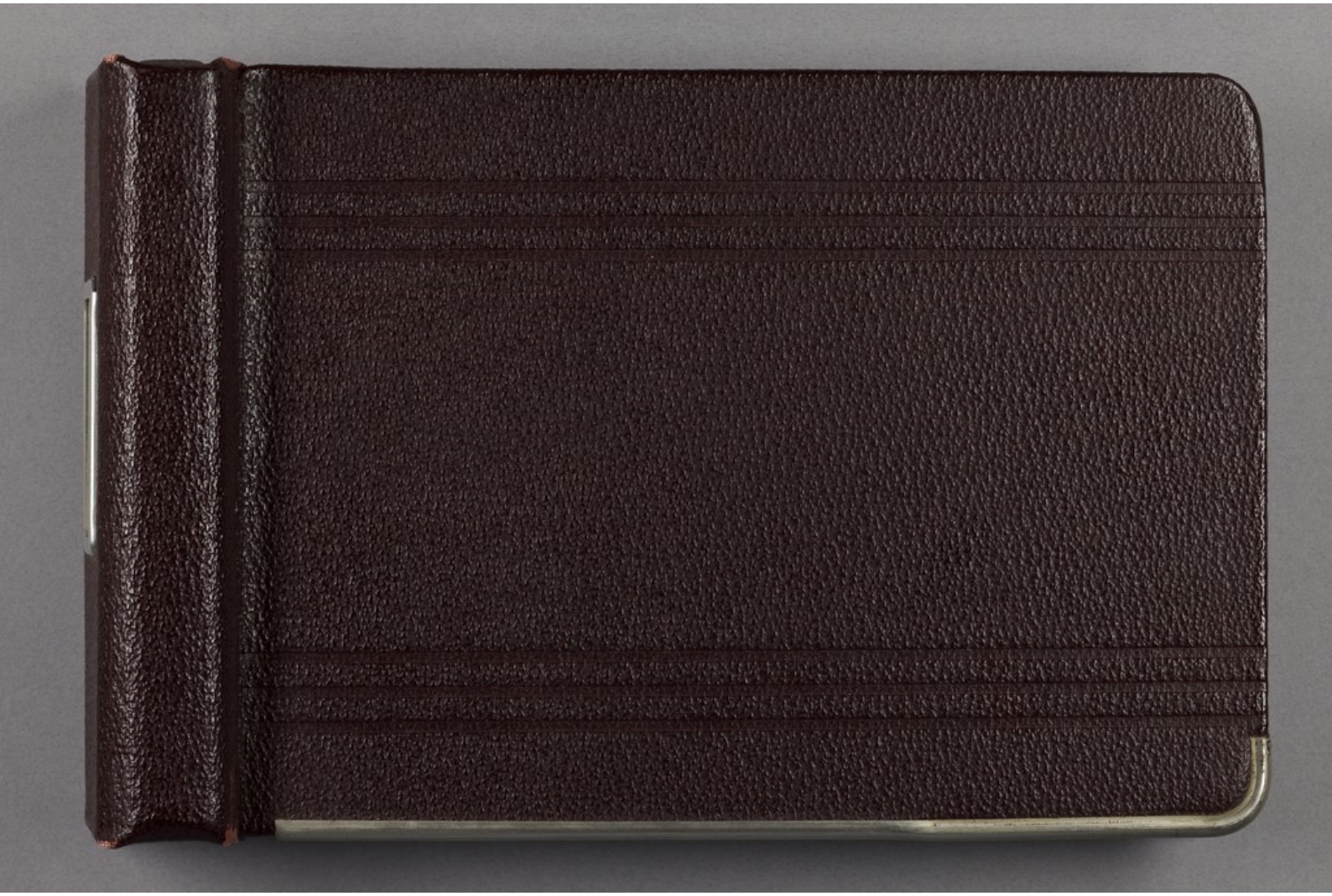


HYB

HYB EXPERIMENTS - INDEX

[Trypan blue: see also ANT-390,391,392,394 and (2nd-set) ANT-396.
For antigenicity of hybrid cells, see ANT-393,397.]

1. Newborn A → adult CBA
2. Newborn CBA/A → CBA
3. Adult CBA/A → A & CBA
4. Newborn CBA/A → adult A
5. Adult CBA/A → A & CBA
6. Adult CBA/A → A & CBA plus trpan blue
7. Adult CBA/A → A
8. Adult CBA/A → A plus trypan blue
9. Adult CBA/A → A & CBA plus appropriate antiserum.
10. Newborn CBA/A → adult A treated with trypan blue
11. Hyperimmunization only
12. 12½-day foetal CBA/A skin → adult A treated with trypan blue.
13. [a] 17½-day foetal hybrid skin → adult A + antiserum
    [b] 17½-day foetal hybrid kidney & lung → normal adult A's.
14. Adult CBA/A → A plus trypan blue and antiserum.

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    [b] 17½-day foetal hybrid kidney & lung → normal adult A's.
14. Adult CBA/A → A plus trypan blue and antiserum.

15. 15/16 day foetal hybrid → adult A + trypan blue & antiserum

16. 14-day foetal hybrid CBA/A → adult A with trypan blue &high (A) or low (B) doses of antiserum; with controls (C).

17. 13-day foetal hybrid as HYB-16: trypan blue only.

18. 14-day foetal hybrid CBA/A → adult A + trypan blue (only) & controls.

19. 15-day foetal hybrid CBA/A →adult A + TB + antiserum; with controls.

20. Control adult CBA/A hybrid skin → C3H/A hybrids.

21. Adult CBA/A hybrid skin → adult C3H/A hybrids + trypan blue.

ADULT CBA/A HYBRID SKIN → ADULT C3H/A HYBRIDS + TRYPAN BLUE HYB-21

[First trial. Controls: HYB-20 q.v.]

10 OCT 1960 Subjects: 5 female and 8 male C3H/A hybrids. Each injected i/p with ≡ 0.3ml M/100 trypan blue.

11 OCT 1960 Graft the 5 +8 mice with CBA/A hybrid male skin, so far as possible with reversed orientation (forwards).

13 OCT 1960 Give the 5 + 8 mice their second standard dose of trypan blue as above.

24 OCT 1960 Inspection. All grafts well healed and 100%

26 OCT 1960 (15) Males: 6 x 100, 1 x 90
Females: 4 x 100, 1 x 100 but very emaciated

27 OCT 1960 (16) Inject all but the sick mouse with 2.0 ml trypan blue (standard dose)
MM: 2 x 100hh, 3 x 100h, 100-h, 90
FF: 100h/100-h/100/90/100-h Last mouse emaciated

18 days: MM 2 x 100hhh / 4 x 100hh / 90
FF 100hh / 100h / 100- / 75hh / 90h (sick mouse recovering)
20 days: MM 2 x 100hhh / 3 x 100hh / 100h / 1 x breaking down (hardening)
FF 100hhh / 100hh / 100h / 50+hh / 90h (sickish mouse)

22 days: MM 4 x 100hhh / 100hh / 100-hh / 0
FF 2 x 100hhh / 100hh / 90 / trace Note that one 100hhh is swollen
The 90 is the still sickish mouse

24 days: MM 100hhh / 75hhh / 75hh / 50hhh / 50-hh / 0 / 0 Mark b.d. in prog. CLIP T

FF 100hhh / 100hh / 90hhh / 90 (sickish) / 0

27 days: MM 25(going)/0/0/0/0
FF 25(going)/0/0/ 100- but this is a bald white graft on the emaciated mouse.

Survivors to date: 20 days = 12, 22 = 11, 24 = 9, 27 = 3, 28 = 1(anomalous) giving an MST of 25/26 days and a much lesser variance than hitherto (hybrid recipients, perhaps).

CONTROL GRAFTS CBA/A → C3H/A HYB-20

[First exp. This is the first time hybrid grafts have been transplanted in the pattern X/Y → Y/Z.]

21 SEP 1960 T.C. grafted 6 male C3H/A hybrids with CBA/A female skin. (Seventh grafts to a female lost.) First inspection at 8 days gave 100% survival in all but 2 is small and about 1/3rd of graft 3 found lost (gnawing?) at 9 days. Score 3 on basis of residual 2/3rds.

graft no:-	9 days	10 days	11 days	12 days	13 days	14 days	15 days
1	100	100		50	0	0	0
2	100	100		100	100	100*	50+
3	100-	100		50	50-	0	0
4	100-	100-		100-	0	0	0
5	100	100		100p	100-ph	10	0
6	100	100-		25	0	0	0

*a stationary & indolent graft; whitish & without hairs (though with matt surface).

+ going very rapidly. Final breakdown 16 days. MST ~13 days, max. 15.5 days.

15-DAY FOETAL CBA/A HYBRID SKIN → ADULT A plus TRYPAN BLUE plus ANTISERUM. WITH CONTROLS. HYB-19

[Foetuses exactly 15 days by timed mating during the day 15 days ago. See HYB-16 for nearest comparable experiment.]

8 JUL 1960 Set up 2 cages of A-mm, A = 8 mice, 25-27g, B = 8 mice, 21½-26g.

TB (1) Inject the 8 A's with the standard dose (≡ 0.3ml M/100) of trypan blue 5 hours before grafting.

Ab (2) Inject the same 8 mice with 0.35ml hyperimmune serum from HYB-11(7) q.v. 2½ hours before grafting (s/c).

(3) Prepare grafts from 3 CBA/A hybrid pregnant females: the foetuses (CBAm × Af) were exactly 15 days. Follow technique of HYB-18 exactly & with great success: the 15-day embryos were very easy to handle after the 14-days. The skin was still quite transparent and pretty well invisible by naked eye on the graft bed.

Graft the 8+8 mice by the usual flotation technique: all grafts were reasonably large and perfectly orientated. Should be successful expt. Note 2 LHS opns (p.c. punctures RHS).

TB 11 JUL 1960 (1) Inject As with standard dose trypan blue as above
Ab (2) 2 hrs later inject each with 0.3ml Ab from HYB-11.

Ab [6] Inject all 8 As with ≡ 0.2ml hyprimmune serum from HYB-11.
Ab [11] Ditto.

22 JUL 1960 [14] Inspections. Grafts technically 100% successful.
A.- 7/8 show survival, mostly 100% and some grafts superlative. 1/8 is probably a breakdown.
B.- 8/8 have healed in, but 1/8 shows total breakdown of recent origin, and possibly a further 3/8 show breakdown of very recent origin. 4/8 surviving, but breakdown is in progress. Good results.

TB. Inject 8 As with 2.0ml (standard dose) of trypan blue.

[15] A.- 2/8 gone (probably very recent); 1/8 breakdown far advanced (kill); 2/8 still softish & well pelted but going; 3/8 only are supergrafts. 5 left running.
B.- 8/8 show total breakdown; all probably in last 2 days: s.t. 13-15 days, with median at about 14 (possibly less).

Ab. Inject the 5 remaining As with 0.35 ml Ab each from HYB-11.

[17] As.- Scores now >100, >100 (two super-grafts still); 90 (weak patch ventrally & some signs of hardening); 25; 0. Four left running.

Ab [19] As.- Scores now >100, <100, <100, 0 (this fourth graft may be scored as surviving to this day). The two grafts scored <100 have hardened areas, but there has been hair growth since 17. Graft >100 is still quite perfect & looks remarkable.
Inject the survivors with 0.4ml immune serum (from HYB-11) each.

[21] As.- One graft (the largest) is quite perfect in every respect. Two may score 25: most of the graft is hardened, but there are small soft viable patches. CLIP all grafts.

(24) As. - One graft still perfect, with fresh hair-growth after clipping
one still with about 25% survival
One x b.d. complete (survival time 22 days).

(25) Aa. As above. The graft showing rather less than 25% survival has a well defined soft and epithelialised patch.

Ab

Inject both mice, each with 0.25ml hyperimmune serum (HYB-11).

(27) One graft still perfect, with growing pelt of hairs
One graft is undoubtedly recovering; it has lost its scabby and hard parts, and the rest is soft, epithelialised, and quite large in area. I don't think that there is any doubt about its authenticity. Some straggly hairs are also present. Inject each with 2ml Trypan Blue.

TB

5 AUG 1960 (28) As above. Inject both animals with 0.5ml each of immune serum (from 2 mice of HYB-11). Recovery of the second graft continuing. Clip the perfect graft.
Make expmt. over to T.C. with instructions for further serum and T.B. injections.

10 AUG 1960 (33) Graft 1. Perfect with good hair crop, swollen in appearance. 6x8mm.
Graft 2. Fully recovered good hair crop tiny scab D: margin, swollen.
Mouse scabby about the nose and ears. 4 x 6mm.

Ab
TB

Inject both animals with 0.2ml each of immune serum, & 1ml. Trypan Blue.

13 AUG 1960 (36) Graft 1. 100⁻h fewer hairs, scabbed on Dorsal & post: margin. 2 tiny scabs centrally, swollen & hard. .worse.

2. 100h Good crop hairs (little thin centrally) little puffy much improved now the better of the two, (nose and ears still scabby).

15 JUL 1960 [38] Graft 1 (originally the super graft) still had a clear 25% surviving epithelial patch at 37, but this has just disappeared, giving a survival time of 37 days pretty exactly. Keep in caseof some spectacular recovery.

Graft 2 (which made the dramatic come-back from 25% survival) is about half the size of a perfect graft, and is domed up with a rim that looks as if native ep were trying to eat into it. It it clearly surviving, however, and has a rather sparse crop of wishy-washy agouti hairs. Clip these fairly short. Score still 100.

[40] Slow contraction and hardening of the surviving graft1 still "100-"

[42] Continuing; yet hair growth since 38, and graft, though domed up, still soft.

-> AB Inject 0.23ml neat serum from HYB-11(13).

[44] Continuing deterioration: graft now soft but swollen as if inflated, & still smaller. Clip away hairs again.

[46] Not much change in the graft; but mouse emaciated and with dry eczematous condition of skin. May have to be destroyed.

[48] Still a small patch of survival; but animal in such poor condition that further survival of not much interest. Kill. Take ST = 49 days.

Summary of this exp:-

15-day foetal CBA/A skin → 8 A-mm with 8 controls (untreated).

Control survival times:

days:	13	14	15
survivors:	7	4	0

plus one other with b.d. probably at 12 days = ST 12-15 days, MST 14 days.

Experimental mice:-

days:	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
survivors:	8	7	6	5	4	4	3	3	3	3	2	2	2	2	2

days:	28	.....	36	37	.....	47	48	49	50
survivors:	2		2	1		1	1	1	0

MEL 22·9
MST 18

TRYPAN BLUE (0.3ml M/100) on days 0,3,14,27,33(0.15ml).
HYB-11 antibody (days,ml) 0(0.35), 3(0.3), 6(0.2), 11(0.2), 15(0.35), 19(0.4), 25(0.25), 28(0.5), 33(0.2), 42(0.23) total 2.98 ml

14-DAY FOETAL CBA/A HYBRID SKIN ⟶ ADULT A + TRYPAN BLUE WITH CONTROL. HYB-18

[See HYB-15,16. This expt resembles HYB-16 except for omission of anttiserum. The foetuses were 14-days aexactly by timed matings. General execution inthis expt much superior to HYB-16. Second controls of foetal skin.]

3 JUN 1960 Subjects: 2 × 6 fully grown A males.

(I) Inject 6 mice with 2ml solution i/p containing ≡ 0.3ml 0.01M trypan blue (still original sample) = group A. Injection at 10 a.m.

(II) Dissect >12 skin grafts from dorsum of trunk region of 14-day hybrid (ex CBAm × Af) foetuses.

Procedure as HYB-16 but greatly improved. Great pains were taken to see that the injection with P&C through a bent no.3 needle genuinely lifted the epidermis right off the subdermal tissue and that the needle penetrated below the skin and not into a deeper plane. In removing the skinit was found necessary to define a bold rectangle with knives & then push & stroke off the skin in one piece. The resulting grafts were excellent, and their orientation (by folding) clear.

(III) Graft the 2 × 6 mice into open style beds not much larger than were approrpriate to the grafts. By applying grafts by pipette in a big drop of saline the orientation showed up well; little bleeding. A very satisfactory series.

4 JUN 1960 Give 2nd dose of trypan blue as above to A's.

15 JUN 1960 Give 3rd dose trypan blue as above to A's.

[One A lost its plaster, and probably the graft with it, some days ago]

17 JUN 1960 [14]

A's - as usual, very difficult to score. Three grafts are clear; one certainly 0 (this was the one that lost its plaster: above); and two are very dubious: probably no grafts.

B's - Three grafts are visible, of which one is rather small. The other three show no signs of ever had a surviving graft, i.e. hve a complete tissue-paper epithelial surface with no trace of dermis or pigmentation. Thus breakdown, if gaft ever took, must have occurred some while ago. Probably primary non-takes. The 3/6 surviving grafts look on the way out.

[15] - no change.

[16] - [A] 2 super grafts, with sprouting hairs. The other 4/6 killed: no grafts detectable, & if any were present they have probably gone. Presumably 1/3 of these does represent a breakdown.

[B] Of the 3 surviving at 15, one has clearly gone; one has nearly gone and the third might be quite a good graft.

[17] - A: some further hair growth. These are solid & well-defined grafts.

B: 1/2 might be scored as a trace, but this is dubious (tattoo effect, possibly). The other 1/2 is clearly surviving: a well defined button with a typical crop of white hair sprouts. Could be interesting: watch.

[19] - A: 2/2 surviving, but one is slightly contracted & hard & shows no progress, and the other is a super graft, supple & well pelted.

B: Probably some survival in the white button graft referred to on day 17; the other has certainly gone and probably went at 17 days. Follow the apparent survivor very carefully.

[22] - A: 2/3 goodish grafts with hair growth since 19; now quite well pelted, though hints of local hardness in both.

B: Hard to make out. Presence of a small well-defined graft with rather fine woolly hair growth would be obvious but for lack of pigmentation. Can only watch.

[24] - A: slight further hairgrowth in both; but also some hint of scabbing in both grafts. Still classifiable as 100% however

B: no change.

→ INJECT the two A's with standard dose (0.3ml M/100) trypan blue.

[26] - A: both show retrogressive changes but still clear survival in both.

B: less certain now that there ever was a graft here.

[28] - A: one graft has <u>just</u> gone; the other seems to have stabilized temporarily, though no further hair growth.

B: existence of graft uncertain: see day 26.

[30] - A: one graft still satisfactory.

[33] - A: Ditto. There has been hair growth since 28. Clip away. Graft decidedly contracted, but still a soft area.

[35] A - Slow progressive contracture & deterioration. Graft within a day or two of total b/d, but still a soft patch of clearly surviving graft.

[36] A - trace survival just allowable: take ST as 36 days.

SUMMARY:- Trypan blue (0.3ml 0.01M) given to A's at 0,1,12,24 days after grafting.

There were probably 3, certainly two primary takes in both series, with survival times as follows:-

A:	??15	28	36	days
B:	16	17	??17	days

13-DAY FOETAL CBA/A FOETAL HYBRID SKIN → ADULT A + TRYPAN BLUE HYB-17

[Embryos 13-day by timed matings, are are younger than those used in HYB-16 & much more difficult to handle. Note no antiserum used here.]

19 MAY 1960 Subjects: 5 A-line ff

(I) Inject all five i/p at 12 am with 2ml solution ≡ 0.3ml 0.01M trypan blue (original sample still).

(II) Dissect 8 embryos by method described under HYB-16, but, because of their smaller size and still tinner skin, lesssatisfactorily. The skin was pretty fragmentary, but probably just usable: the actual process of detachment of the 'blown up' skin was not completely mastered.

(III) Graft the 5 mice with 2-3 fragments each by method of HYB-16.

Grafting very unsatisfactory: orientation was almost impossible, though this was partly because the opns were done at 2.30pm before the hosts had time to blue up adequately to make a dark background. One mouse died, and only 4/5 have recived trypan blue.

In general, the 13-day embryos shoukd be just workable as skin graft donors if attention is paid to allowing the hosts to blue up and if somewhat larger skin areas can be dissected away.

20 MAY 1960 2nd inj ≡ 0.3ml 0.01M trypan blue.

[15] One mouse had died. Of the three which had been treated

with trypan blue, only 1/3 shows a small smooth button of surviving graft, at present bearing no hairs. One mouse not treated by trypam blue had no surviving graft.

[16] The 1/3 trypan blue treated mouse still has perceptible graft. Give standard dose of trypan blue.

[18] Dispose of 2/3, definitely not graft-bearing. The 3rd mouse may have a graft, but it is not bearing hairs & pigmentation cannot be detected aginst the blue background.

[20] The graft-like appearance of the apparent survivor is slowly disappearing.

[The mouse was followed for 2 more weeks: no further development occurred; either it never bore a graft or it brokw down ~15-16 days.]

14-DAY FOETAL CBA/A HYBRID SKIN —> ADULT A + TRYPAN BLUE & ANTISERUM (HIGH & LOW DOSAGE). CONTROL. HYB-16

[Second trial: cp. HYB-15. The embryos here are definitely 14-day by timed mating, and were younger than those used in HYB-15. OK also by Gruneberg chart. For antiserum alone, see HYB-13A. First controls with foetal skin in this series.]

13 MAY 1960 Subjects: 3 × 5 fully adult A-line males.

(I) Inject 2 × 5 at 11 a.m. with 2ml solution containing ≡ 0.3ml 0.01M trypan blue. [This is the original sample still, kept in refrigerator in diluted form.] These constitute A,B.

(II) Dissect 15 skin grafts from dorsum of back of 14-day CBA/A hybrid embryos aged by timed matings (CBAm × Af).

Procedure. This improved as time went on, and following gave good results which should be applicable even to 13-day embryos. Slip 30-gauge needle, bent to a right angle, under dorsal skin of embryo in several places, and with tuberculin syringe inject either P&C or (a few expts) air so as to lift the skin off the body. The results are unmistakeable when the injection is properly executed. Then cut the trunk region to define a large rectangle symmetrically over dorsum of trunk, and with this lump lying belly side down coax off the loosened skin. The grafts are thin, delicate and rather gelatinous, and are almost invisible on a normal graft bed (contrast skin in HYB-16).

(III) Graft 3 × 5 A-line mice into small open style beds. First put a drop

ortwo of P&C on the bed, then transfer graft by needle or pipette, and orientate so far as possible by taking advantage of the natural curl of the skin, which is inwards (outer surface convex). Unfortunately the grafts were almost invisible in the C beds (no trypan blue), with corresponding uncertainty of orientation.

(IV) Bleed 4 × HYB-11(8) mice for 2ml antiserum anti-CBA. Dilute to 6ml. Inject 5 mice of group A with 1.0ml each. Dilute the remaining 1ml to 5ml, and inject the 5 mice of group B with 1.0ml each. All s/c.

Summary: 14-day hybrid foetal grafts on

A. 5 A-mm receiving each ≡ 0.3ml 0.01M trypan blue + 0.33 ml antiserum
B. - - - - - - - + 0.067 -
C. - - untreated.

Notes: Both the dissection & the grafting procedures improved in course of the expt. As the mice were done in the order C-B-A, the A grafts are ofa good size and properly orientated; the B's smaller & more ragged, but probably well orientated; and the C's are defimitely ragged and only dubiously orientated. Thus breakdown in C should not be assumed unless the grafts become recognizable as such.

TB 14 MAY 1960 Give the 10 mice of A B their second injection i/p of 2ml solution ≡ 0.3ml 0.01M trypan blue.

AB 16 MAY 1960 Get hyperimmune antiserum from HYB-11, & inject the 5 A's with 0.27ml each and the 5 B's with 0.054 ml each.

AB 20 MAY 1960 Inject 5 A's with 0.31ml immune serum from HYB-11, and the 5 B's with 0.063ml each. The A's look scruffy & there has been severe loss of wt in 2/5.

AB 23 MAY 1960 Inject 5 A's with ≡ 0.25ml immune serum & 5 B's with ≡ 0.05 ml immune serum, from HYB-11.

[Loss of weight & general scruffiness of A's, in spite of same dose of trypan blue, is disturbing.]

27 MAY 1960 [14] First inspection.

[A] 2/5 definite (one small), 1/5 possible; 2/5 apparently non-takes: kill

[B] 1/5 good, 1 definite but small, 1 possible (but ? b/d), 2/5 very dubious. Keep all going.

[C] 3/5 definite non-takes (clear against pink background); 2/5 definite good takes with 14 day survival. Keep only these 2 going.

NOTE: rather unsatisfactory. It is possible that the apparent duds in A,B were written off too quickly, the pigmentation being impossible to discern against the blue background. Be more cautious in future. Continue injections for the time being.

Surviving animals: A = 3 B = 5 C = 2.

AB Inject the A's with 0.25ml and the B's with 0.05ml immune serum from HYB-11.

[15 DAYS] Pattern now a little clearer. In A, 2/3 good & well defined; 1/3 there, but graft is acbby (picking yesterday) and the animal is scraggy & scruffy - omit from TB injections. In B, 1/5 clear dud now

discarded, leaving 4; of these, 2/4 clear, 1/4 clear but micro-sized; 1/4 still very dubious. In C, 2/2 OK. Note hair bristles (yellow points) on all grafts.

TB Inject 4/4 B's and 2/3 A's (omitting 1/3 = Eo referred to above) with the standard dose (≡ 0.3ml 0.01M) trypan blue.

16 days. 1/2 of C have gone and the other 1/2 is going &may also have gone.

The A's & B's are unchanged, but are still as difficult to interpret as ever. Probably only 2/4 of the B's are genione graft takes.

17 days. Breakdown now certain in the other 1/2 of A: hence take survival times as 15 & 16 days.

A's and B's continue unsatisfactory: only one in each is a clear & well defined graft. Watch.

Note that there has been no further hair growth on or expansion of any graft. The general appearance is frankly one of retrogression.

AB Inject the 3 A's with 0.25ml & the four B's with 0.063ml immune serum from HYB-11 q.v.

18 days. There are detectable grafts in only one each of A & B, andthese seem quite clearly to be regressing.

19 days. Kill all B's and all but one of the A's: in this last A it is still not certain that b/d is complete, though there has been no further hair growth.

20 days. Breakdown now certain in the last A: kill. Expt concluded.

15/16-DAY CBA/A FOETAL SKIN → ADULT A PLUS TRYPAN BLUE & ANTISERUM HYB-15

[First trial of foetal skin + bth trypan blue & antiserum. Antiserum alone: HYB-13A; trypan blue alone (12½ days embryos): HYB-12.]

29 FEB 1960 (a) Inject 6 large A-mm with 2ml solution containing 0.3ml 0.01M trypan blue. Injection 4½hr approx before grafting.

(b) Get dorsal skin grafts from foetuses of A-f pregnant by CBA male.

> The embryos were not ageable by timed mating. By Gruneberg chart the open pigment ring structure of the eyes suggested 14+ days, but the size of the embryos and the the pattern of the integument was more like 15+ days. At all events, the grafts were much younger than HYB-13A though much older than HYB-12. Dissection under binocular was easy, the orientation is probably correct, and the grafts were of a good size. There was no question of removing the immediately subdermal layers: the whole integument is grafted. Embryos somewhat younger than this should be dissectable with success: the skin here was fairly firm and elastic and handled very well. The area removed corresponds to a good 2 square inches on an adult.

(c) Graft to open areas as usual RHS on the 6 mice.
(d) Just after recovery from anaesthesia inject all mice s/c with 0.3ml each immune serum from HYB-11(4).

2 MAR 1960 [2] Reinject all mice with ≡ 0.3ml 0.01M trypan blue.

4 MAR 1960 [4] One mouse found dead: trypan blue?
Inject the 5 remaining mice s/c with ≡ 0.30 hyperimmune serum from HYB-11 q.v.

7 MAR 1960 [7] Inject all 5 s/c with 0.32ml immune serum from HYB-11.

12 MAR 1960 [12] INSPECTION OF GRAFTS. 3 excellent grafts: thick, pigmented, with hair growth [note that grafts include full thickness of integument]. One graft represented only by a small button, but perfectly usable. The fifth a doubtful take: watch.

Inject all five mice s/c with 0.6ml fluid containing 0.27 ml hyperimmune serum from HYB-11, q.v.

[13] 3 x 100: perfect grafts. 1 x trace graft looks smaller than ever. The fifth may well have no surviving dermal tissue & probably will have to be discarded.

[14] No change, but discard the fifth animal which clearly has no surviving graft dermis.

Inject the four remaining mice with 2.0ml solution containing the standard dose of trypan blue (0.3ml 0.01M).

15 MAR 1960 [15] The graft represented by only a minute ventral tag simply came off: possibly genuine breakdown: kill.
The 3 remaining grafts clipped. They have a dry scurfy appearance

with hints if marginal weakness, and may well be om way out. But note that thickness may simply mean differentiation of panniculus and fatty layers.

→→ Inject the three remaining mice with 0.25ml each hyperimmune serum from HYB-11(6).

16 MAR 1960 (16) 1 x 100% with pelt re-growing (?), rather thick but still soft
1 x high degree of survival but rather pulpy
1 x some survival, with small superficial scabs and hardening

18 MAR 1960 (18) Much as above, but slight deterioration in all
REINJECT each mouse s/c with 0.25ml hyperimmune serum from HYB-11(6).

19 MAR 1960 (19) As above

22 MAR 1960 (22) 1 x 100 with thick pelt, graft very thick but still fairly supple
1 x high degree of survival but hairs now scruffy, graft very palpable and hardening
1 x very small graft, hardening and very button-like - probably still some survival

REINJECT each mouse S/C with 0.25ml hyperimmune serum (HYB-11(6)

25 MAR 1960 (25) All 3 grafts showing the typical symptons of a slow reaction - swelling, scurfiness, hardness etc. Difficult to score with accuracy, but 2/3 still with pretty high degree of survival, the 3rd probably with some.

Reinject each mouse with 0.25ml hyperimmune serum from HYB-11(7).

29 MAR 1960 (29) Grafts still hanging on - b.d. is extremely tardy and 2/3 have quite definite survival, even though the precise degree is impossible to ascertain. The small graft is a pretty bad way, but impression is that there is still some survival.

Reinject each mouse with 0.25ml s/c hyperimmune serum from HYB-11(7).

1 APR 1960 (33) 2/3 continue to deteriorate but clearly still show some degree of survival. The epithelium is distinctly visible through the thinning pelt and there can be no doubt of its presence.

The 3rd graft (small) must now be considered to be out of the race ..... survival time of approx. 31 days.

Reinject the remaining 2 mice with 0.25ml s/c hyperimmune serum from HYB-11(7). Keep the 3rd mouse for further observation.

5 APR 1960 (37) The grafts are now rapidly going - one has scabbed very severely over the week-end and probably has no more than trace survival this day. The second is showing minor scabbing, extreme scurfiness and baldness. Both grafts are now quite hard and palpable.

Rough scoring: 1 x trace
1 x some but still definite survival
1 x 0

Decide not to reinject these mice - would be waste of serum.

8 APR 1960 (40) Definite breakdown confirmed in another graft ------
survival score of approx. 38 days
The last graft is very hard and scabby, but has conceivably slight epithelial survival.

Note that animals, although quite lively, look very scruffy - tendency towards cephalic baldness, and scabbing on ears and nose. Long range Trypan Blue effects??

SUMMARY: This foetal skin usable as skin. Try younger still.

Trypan blue, 0.3ml M/100 at days:- 0, +2, +14
Antiserum at days (dose ml):- 0(0.3) 4(0.3) 7(0.32) 12(0.27)
15(0.25) 18(0.25) 22(0.25) 25(0.25)
29(0.25) 33(0.25) Total: 2.69 Mean: 0.27

Survival times: [One mouse died at 4 days]; 2 non-takes or virtual non-takes leaving three, lasting >22<25, ~37, ~38, suggesting significant prolongation vis-a-vis adult hybrid skin.

[PTO

Note that these foetal graft bearers received one injection less trypan blue and slightly less mean antiserum; nevertheless the survival scores compare as follows:-

Adult CBA/A: HYB-14	>14	18	>>25, >25	38		days
Foetal CBA/A: HYB-15			>22<25	37	38	days

This just justifies going to still younger foetal skin.

ADULT CBA/A HYBRID SKIN → ADULT A WITH TRYPAN BLUE & ANTISERUM HYB-14

[First trial of the combination. Controls: HYB-3,5,7. Antiserum only: HYB-9. Trypan blue only: HYB-6,8. Both treatments give some prolongation (much more with trypan blue than antiserum).]

18 FEB 1960 (1) At 10.0 a.m. inject six A-mm with the standard half-dose of trypan blue (≡ 0.38ml 0.01M trypan blue in phosphate buffer 0.2M pH 6.9.

(2) At 2.0 p.m. inject all six s/c with 0.6ml hyperimmune antiserum (anti-CBA/A hybrid) from HYB-11 q.v.

(3) At 4.0 p.m. transplant one middle-sized adult female hybrid skin graft to each mouse. Grafts from a pregnant female (hybrid X hybrid: F_2 foetuses); grafts were inactive but came from areas near moderately active patches.

20 FEB 1960 Give all 6 mice their second injection ≡ 0.15ml 0.01M trypan blue.

22 FEB 1960 Give all mice 0.26ml hyperimmune antiserum from HYB-11 q.v.

25 FEB 1960 Give all mice 0.26 ml hyperimmune serum from HYB-11 q.v.

27 FEB 1960 = 9. One mouse died, leaving 5 only.

29 FEB 1960 Give all mice 0.30ml hyperimmune serum from HYB-11.

Inspection[11]. Well healed grafts. At first inspection, 5 x 100; but 2/5 showed deterioration after unbandaging, and these are only 50-75% in score.

[12] 100 100 90 90 50-

[13] 100 100 75 50 25. Inject standard dose (0.3ml 0.01M) trypan blue.

[14] Death of mouse scored 75 yesterday: still 75. Remaining scores unchanged, viz: 100 100 [75] 50 25.

[15] Scores 100 100 50 25 as before: hair growth has just started in the 2 100's.

Inject each mouse s/c with ≡ 0.25ml hyperimmune serum from HYB-11 q.v.

[16] 100 100 50 25- . Some further hair growth.

[17] 100 100 25 10

[18] 100 100 25 10. Progressive hair growth in 1/2 100's: in the other growth is now stationary.

Inject the two 100's only with 0.32ml immune serum from HYB-11

[19] 100 100 25 0

[20] 100 100 25 0. Further hair growth in 1/2 of the 100's.

[21] 100 100 25 0.

]22] 100 100 25+ 0. There is no doubt about the 25. 1/2 100's looks stable; the other is scurfy & may be going rapidly.

12 MAR 1960 [23] One graft is excellent; one is contracting and hardening; the third is just holding on without hairs in a more or less stationary state. This third mouse in poor shape. Scores 100, 100--, 25.

[23, cont.] Inject the 2 better graft-bearers (as before) with ≡ 0.27 ml hyperimmune serum.

[24] 100, 50cc (hardening & contracting rapidly), 25. The 100 is still a perfect graft, though hair growth is sparse.

[25] The graft which was 100 at 22 days is now clearly 0. The "25" graft seems to have healed over to give a complete surface, and would now be scored 100---; it is not likely to grow hairs, however, and therefore not worth wasting antiserum on. Kill.

The one 100 graft continues perfectly soft and supple.

→→ Inject this one mouse with standard dose of trypan blue, i.e. 2ml solution containing ≡ 0.3ml 0.01M trypan blue.

[Note on MST. One mouse with a 75 graft died at 14; one mouse killed with a surviving graft at 25. There have been two genuine breakdowns, at days 19 and 25. MST can be taken as greater than 25.]

15 MAR 1960 [26] The one surviving graft perfect, and animal in good shape. Clip the graft; note hair distribution not perfectly even, and some greyish hairs.

→→ Inject with 0,25ml hyperimmune serum from HYB-11(6).

16 MAR 1960 (27) Graft unchanged

18 MAR 1960 (29) Graft unchanged - still perfect and with slowly regrwoing hairs. Possibly slightly scurfy, but soft and supple.

Reinject with 0.25ml hyperimmune serum from HYB-11(6)

22 MAR 1960 (33) Graft still in pretty good shape, but a little scurfy, hairs somewhat scruffy, and possibly suffering from contracture

Reinject with 0.25ml hyperimmune serum from HYB-11(6&7)

25 MAR 1960 (36) Graft may be beginning to down hill - hair very thin and surface definitely scurfy.

Reinject with 0.25ml hyperimmune serum from HYB-11(7)

28 MAR 1960 (39) Breakdown of the graft has been quite dramatic over the week-end - loss of epithelium showing up dark blue (almost black) dermal surface. Breakdown just about complete.

29 MAR 1960 (40) Confirm breakdown of graft - kill mouse.

SUMMARY:

Trypan blue (0.3ml M/100) given at days 0, +2, +13, +25

Antiserum (dose ml) given on days:- 0(0.60) 4(0.26) 7(0.26)
11(0.30) 15(0.25) 18(0.32)
23(0.27) 26(0.25) 29(0.25)
33(0.25) 36(0.25)

Total 3.26 mean 0.30

Survival times: >>14 (died)
18,25
>>25 (killed: a poor graft)
38

Thus MST probably >25, with some evidence of synergistic action between trypan blue & antibody.

17½ DAY FOETAL HYBRID SKIN → A LINE MICE plus ANTISERUM
17½ DAY FOETAL LUNG & KIDNEY → normal ADULT A MICE

HYB-13

8 FEB 1960 Antiserum. Bleed 5 HYB-11 A-line mice and (by mistake) 1 HYB-11(2) A-line mice; get 3.6ml clear serum.

Donor tissues: remove dorsal skin, kidneys and lungs from pregnant A-mouse with 17.5 day (Gruhberg scale) CBA/A hybrid embryos. Trim the skin carefully: grafts of normal size (except one small graft).

[A] Inject 6 A-mm with 0.6ml each hyperimmune antiserum from HYB-11 donors. Graft each 90 min later with 17½ day foetal hybrid skin RHS. Graft a seventh mouse (PIC) which received no antiserum.

[B] Implant 7 A-mm with one foetal hybrid kidney (whole) RHS and about half a foetal hybrid lung (LHS). So far as possible the grafts lay in the standard skin position between panniculus and dermis (but some will have penetrated). A few grafts may not have come out of the trochar. Technique was to make posterior incision, followed by tunnelling with MM forveps and inseryion by rather large trochar.

11 FEB 1960 [3] Get 1.6ml hyperimmune serum from 3 x HYB-11 A-lines; dilute to 3.6 ml and inject the six mice of group A with 0.6ml = 0.26ml immune serum each s/c.

15 FEB 1960 [7] Get 1.4ml hyperimmune serum from 3 HYB-11 A-lines; dilute to 3.6ml and inject the six mice of group A with 0.6ml = 0.23 ml immune serum each s/c.

16 FEB[8] Rebandage 2 mice. Of these, one had a partially pigmented & well healed graft. Other graft may be a partial loss through muck getting under plaster:watch.

18 FEB 1960 Inspection [10]. The PicH animal (no antiserum) has a small pigmented fragment, perfectly scorable.

Of the 6 injected mice, 1/6 has only a minute trace of pigmented epithelium underlain by dermis: kill. Of the remaining 5, 3/5 are good biggish grafts, well thickened, with hairs just piercing; 2/5 are smaller with, so far, only deep pigment shadowing.

Immune serum - Inject the 5 remaining mice with 0.25ml immune serum each from HYB-11 and 11(2).

[12] Most grafts show progressive differentiation with hair growth. 5/5.

[14] The grafts have been deteriorating rapidly. There are now 3/5 (possibly only 2/5) survivors: all are hardish and somewhat contracted. There is the usual difficulty about determining end-points. No injection of antiserum.

[15] Total breakdown is now certain. Take MST as 14 days roughly -- perhaps only 13½. Evidently antiserum has had no effect.

4 MAR 1960[25] Inspection of Group B. There was no sign of the implanted kidney and lung grafts when animals were opened and graft area of integument examined from inner surface.

12½ day FOETAL CBA/A SKIN → ADULT A'S TREATED TRYPAN BLUE HYB-12

[Previous foetal hybrid skin expt: HYB-13]

10 FEB 1960 Inject 6 A-mm with 2ml 0.15M NaCl containing .030ml ("half dose") of 0.01M trypan blue.
[One died under anaesthesia at opn, leaving 5].

11 FEB 1960 Dissect skin from 12½-day CBA/A hybrid foetus (A-female). Age known from timed mating and Gruneberg chart. After various less satisfactory procedures, adopt the following: inject head region as close below skin as possible with P&C through a bent no.30 needle, so lifting the excessively delicate skin from the head. Now slice off the dome of the head and tease away the brain tissue as far as possible, leaving a somewhat crumpled and contractedskin mebrane. Three of the ppns were very dirty, and five reasonable clean. Orientation probably OK but one can't be certain.

Graft to standard sized open areas on the 5 trypan-blue treated A line males. Two mice received two grafts each; the others one each. If orientation was all right in the dish it will be all right onthe graft bed. Open areas were used to prevent loss by premature contracture before the skin becomes 'solid'.

This expt is purely exploratory, to see if 12½ day foetal skin can in fact be grafted.

13 FEB 1960 Reinject the 5 mice with ≡ 0.03ml 0.01Mtrypan blue.
22 FEB - One mouse found dead, leaving 4.

22 FEB 1960 [11] Plasters off the 4 mice. 1/4 shows a corner tag of what might be a surviving graft. None of the others show anything graft-like, though much of the epithelium may be of graft origin. The beds are uncontracted and fully surfaced.

25 FEB 1960 [14] Still no signs of graft take, though there may be a fragment in one of the four. Reinject with ≡ 0.03ml 0.01M trypan blue.

29 FEB 1960 [18] 1/4 shows a dorsal fragment of what appears to be surviving graft tissue (this animal is in poor shape). Nothing visible on the others.

4 MAR 1960 [22] There is no sign of graft take: destroy the 4 mice.

A & CBA MICE HYPERIMMUNIZED BY CBA/A HYBRID LYMPHOID CELLS HYB-11

Immunization schedule. HYB-11 = 23 A-males & 16 CBA-ff, the latter from a suspicious stockpot in which there had been some deaths.

16 JAN 1960 Get 340 lymphoid tissue (spleen, nodes, some Peyer's patches, thymus) from 2 CBA/A hybrid ff. Render through sieve into 55ml P&C and count at 1/5 (14 fields) = 34.6 mean = 21.5 m/ml nucleated cells, representing a yield of 348m/100mg or about 35m/mg wet weight.

Inject the above mice with 1.0 ml each i/p = 6.3mg = 21.9m cells. Use rest for ANT-395 booster injection q.v.

23 JAN 1960 Get 268mgm lymphoid tissue (the usual, but no Peyer's patches) from 2 CBA/A hybrid mice, m & f. Press through sieve into 20ml P & C, and count: 51½ mills ∅ ml. Dilute to 20ml.

Inject the 39 mice i/p with 0.5 ml each. Dose = 6.8mg = 12.8m cells.

Note that the dose of cells / mgm of tissue is much lower than that obtained by PBM above. Why?

30 JAN 1960 There remain 18 As and 14 CBAs. To these add the following mice distinguished as HYB-11(2): 6 CBAmm = ANT-395C q.v., have received antigen and A-line homos removed at 6 days; 6 Aff (unused mice). 44 mice.

Get 0.46g lymphoid tissue from 3 CBA/A hybrids and dilute to 46ml PC/2. Inject mice with 1.0ml ≡ 10mg/mouse (the As of HYB 11(2) got about 14 mg/mouse).

6 FEB 1960 Add in HYB-11(3) = 6 CBAff & 6Aff. Totals now: HYB-11 = 17A &14CBA, todays injection 4th; HYB-11(2), 6A + 6CBA, 2nd; HYB-11(3),6A + 6CBA, 1st.

Inject all with lymphoid cells from 10mg lymphoid tissue each (3 young CBA/A hybrid donors: spleen, nodes, thymus = 570mg).

8 FEB - bleed 5A's from HYB-11 and (by mistake) one A from HYB-11(2).

11 FEB - bleed 3 further HYB-11 A's for HYB-13 q.v.

13 FEB 1960 ADD in HYB-11(4) = 8 A-ff, unused. Inject all 54 mice with 1.0 ml P&C i/p containing lymphoid cells from exactly 10mg wet wt spleen.

15 FEB - Bleed 3 As from HYB-11; use for HYB-13A.
- Bleed the remainder (6) of the HYB-11 A's and 4/5 of the HYB-11(2) A's for use under HYB-13 & 14 q.v.

20 FEB 1960 ADD IN HYB-11(5) = 8 A-ff, unused. Inject all 49 mice with P&C suspension containing 10 mg/mouse as before.

22 Feb - Bleed last mouse of HYB-11(2) and 2 mice of HYB-11(3) for HYB-14 q.v. All mice are A's.

25 FEB 1960 - Bleed 3 A's of HYB-11(3) for HYB-14 q.v.

IMMUNE SERUM I

27 FEB 1960 HYPERIMMUNE SERUM SAMPLE I. Bleed the 14 CBA's of HYB-11: these have had 6 weekly injections of 10mg wet weight hybrid CBA/A tissue & are being bled 7 days after the last injection. [Actually injj 1,2 were of only 6.3 & 6.8mg respectively]. Allow clot to retract, spin well, mix thoroughly, and ampoule in measured 0.25ml aliquots = SERUM I.

Remaining mice: Add in HYB-11(6) = 8 unused A-ff. Inject all 37 mice with 10mg wet weight hybrid CBA/A spleen-thymus cells.

29 FEB 1960 - Bleed the last A of HYB-11(3) + 2A's of HYB-11(4). Use: HYB-14.
- Later bleed 3 more HYB-11(4): use, HYB-15.

4 MAR 1960 Bleed 3 HYB-11(4) and 2 HYB-11(5) for use under HYB-14,15. Then add in HYB-11(7) = 8 A-ff as before, and inject all remaining mice (12 CBA & 22 A's)with 10mg wet wt lymphoid tissue from CBA/A hybrid donors as usual.

7 MAR 1960 - Bleed 4 HYB-11(5) for HYB-14,15.

12 MAR 1960 (1) Bleed the two remaining A's of HYB-11(5) plus 2 A's of HYB-11(6), and inject serum into HYB-14,15 q.v.
(2) Set up the following extra stocks: HYB-11(8) = 8 A-mm, and

HYB-11(9) (2 pots) = 8 + 8 CBA-ff.

(3) Inject all the above mice with ≡ 10mg wet weight CBA'A hybrid lymphoid tissue.

IMMUNE SERUM II

(4) IMMUNE SERUM SAMPLE II. Bleed the 12 CBA mice comprising HYB-11(2) (males) and HYB-11(3) (females). Spin for serum and store in 1ml ampoules in 0.25ml volumes, carefully measured.

These mice have had six and five respectively weekly injctions of ≡ 10 mg wet wt CBA/A hybrid lymphoid tissue, and have been bled eight days after the last injection.

Remaining mice:- HYB-11(6) = 6 Aff
11(7) = 8 Aff
11(8) = 8 Amm
= 38 mice.

HYB-11(9) 8 + 8 CBA-ff

15 MAR 1960 Bleed 2 HYB-11(5): use as under HYB-14, 15.

19 MAR 1960 Inject remaining mice with 10mgm wet weight CBA/A hybrid lymphoid tissue

27 MAR 1960 Ditto

1 APR 1960 Ditto

8 APR 1960 There now remain: pot 8 = 8 Amm
pot 9 = 8 + 8 CBA-ff
The rest have been used for injection of immune serum into HYB-14 & 15.

9 APR 1960 Inject the above mice with 10mgm wet weight lymphoid tissue from CBA/A hybrids, i/p.

18 APR 1960 There remain:

8As = HYB-11(8) 5 weekly injections so far
16CBAs = HYB-11(9) ditto

Add: 6 + 5 further CBAs (ex HAEM-8 q.v.) = HYB-11(10), 2 pots.
8 + 8 further As = HYB-11(11), 2 pots.

INJECT all the above mice with ≡ 10mg wet wt lymphoid tissue from CBA/A hybrids as usual.

27 APR 1960 Set up HYB-11(12) = 8 A-mm.

Omitting HYB-11(8) & (9), which have had 6 injj already, inject all mice with CBA/A hybrid lymphoid tissue (≡10mg/mouse as usual).

3 MAI 1960 Inject all mice with ≡ 10mg CBA/A lympoid cells.
Note that HYB-11(8) have now had 6 + 1 weekly injections.

10 MAY 1960 Inject all 45 mice with suspension ≡ 10mg CBA/A hybrid lymphoid tissue.

13 MAY. Inject HYB-16A,B with antiserum from 4 × HYB-11(8) mice.

16 MAY 1960 Set up HYB--11(13) = 10 A-line males.

Leaving out the 4 remaining mice of HYB-11(8), inject the remaining 47 with ≡ 10mg CBA/A hybrid lymphoid tissue. HYB-11(13) get 20mg.

16 MAY. Bleed 3/4 HYB-11(8) for HYB-16A,B.

20 MAY. Bleed 1/1 = last HYB-11(8) and 2/16 HYB-11(11). See HYB-16A,B.

23 MAY. Bleed 3/6 in Pot 1 of HYB-11(11) for HYB-16 q.v.

23 MAY 1960 Inject all remaining mice except the CBA's with ≡ 10mg CBA/A hybrid lymphoid cells.

27 MAY. Bleed 2/11 HYB-11(11); use for HYB-16 q.v.

30 MAY 1960 Set up HYB-11(14), 2 pots, 10 + 9 CBA-ff.

Inject all mice except 2/9 HYB-11(11) with ≡ 10mg hybrid lymphoid tissue. (The largest mice of the new pots got 15mg as a starter).

30 MAY. Bleed 2/9 HYB-11(11); use for HYB-16.

13 JUN 1960 Inject all mice with ≡ 10 mg CBA/A hybrid lymphoid tissue.

Remaining mice with number of weekly 10mg injections:-

HYB-11(11)	7 left	1 1 1 1 1 1 1 0 1	= 8
11(12)	8	1 1 1 1 1 1 0 1	= 7
11(13)	10	1 1 1 0 1	= 4
11(14)	10 + 9	1 0 1	= 2

20 JUN 1960 Inject all mice with ≡ 10mg hybrid tissue as above.

27 JUN 1960 Add HYB-11(15) = 6 + 6 CBAff. Omit HYB-11(11) & 11(12) from this injection. Otherwise give HYB-11(13) & (14) 10mg dose and 11(15) a starter of 20mg.

4 JUL 1960 Inject all mice with ≡ 10mg hybrid splenic tissue. Record now:-

HYB-11(11)	7 mice	1 1 1 1 1 1 1 0 1 1 0 1	= 10
(12)	8 -	1 1 1 1 1 1 0 1 1 0 1	= 9
(13)	10	1 1 1 0 1 1 1 1	= 7
(14)	10+9	1 0 1 1 1 1	= 5
(15)	6+6	1 1	= 2

8 July Bleed 5/7 HYB-11(11) for HYB-19
11 July Bleed 2/2 HYB-11(11) and 2/10 11(13) for HYB-19.

11 JUL 1960 Inject all remaining mice with ≡ 10mg CBA/A hybrid spleen & thymus cells.

12 JULY Graft 8 mice of the 10 in HYB-11(14): see ANT-415.

14 JULY Bleed 3/8 HYB-11(12) for HYB-19.

18 JULY Biopsy the grafts done 12 July and bleed all HYB-11(14) for hyperimmune serum. See ANT-415 for details.
<Note that this is 7 days after the 6th of 6 weekly injj of ≡ 10mg CBA/A hybrid tissue.>

19 JULY Bleed 3/5 HYB-11(12) for HYB-19.

19 JUL 1960 Inject all remaining mice with ≡ 10mg CBA/A hybrid lymphoid cells. Scores now:-

HYB-11(12)	2 mice	11 injj
11(13)	8	9 injj
11(15)	6+6	4 injj

23 JULY Bleed 2/2 HYB-11(12) & 1/8 11(13) for HYB-19.

27 JULY Bleed 2/7 HYB-11(13) for HYB-19.

29 JUL 1960 Inject remaining mice (5 × 11(13), 6 + 6 11(15)) with ≡ 10mg CBA/A hybrid spleen tissue.

16 AUG 1960 Give booster dose ≡ 10mg CBA/A hybrid tissue to the 12 CBA mice of HYB-11(15). Total injections thus 5 + 1.

19 AUG Bleed last A-line mouse (HYB-11(13)) for HYB-19.

25 Aug Bleed 2/12 HYB-11(15) for ANT-419 q.v.

31 Aug Skin-graft 10/10 HYB-11(15): see ANT-421.

6 Sept Biopsies: see ANT-421. One death (ascites tumour)

7SEP 1960 Re-inject the remaining 9 mice of 11(15) with ≡ 10mg CBA/A Hybdrid tissue.

[illegible] Sep 1960 Re-inject the remaining 6 mice of 11(15) with 10mgm CBA/A hybrid tissue.
(3 mice used in abortive ANT-422 expmt).

3 OCT 1960 Re-inject the remaining mice of HYB-11(15). 9th injection.

10 OCT 1960 Bleed put the remaining four mice of HYB-11(15): store 3 × 0.25ml and use 025ml as under ANT-425 q.v. Schedule of injections:-

Days:	0	7	15	22	32	50	72	84	108
mg:	20	10	10	10	10	10	10	10	10

Bled 7 days after last injection. Mice perfectly healthy.

→→ END of series HYB(11) ←←

Newborn A/CBA skin on adults A mice treated with trypan blue HYB-10

[Newborn CBA/A to CBA & A: see HYB-2,4; newborn A→CBA, HYB-1. Present expt is first trial with trypan blue.]

11 JAN 1960 Dilute 30ml warm 0.01M trypan blue in 0.10M phosphate pH 6.9 to 200 ml with normal saline. Inject 14 A-mmm with 2.0ml (≡ 0.3ml 0.01M trypan blue) mixture i/p.

12 JAN 1960 Take skin grafts from 7+4 newborn CBA/A hybrids born overnight from Af x CBAm. Trim. Three of the largest grafts (1 mouse = 1 graft) were subdivided, giving 8 large (? too large) and 6 normal-sized grafts. Transplant in usual way to the 14 treated A males. One death (accidental).

13 JAN 1960 Inject the 13 remaining mice with 2.0ml trypan blue as used 11 January.

22 JAN 1960 (10) All mice in quite good shape
Pot 1: 7 x 100; well healed, pigmented, and with incipient hair-growth over largish areas.
Pot 2: 6 x 100; ditto.

23 JAN 1960 (11) General note on grafts: they are rather thick and spongy (as 11-day mouse skin should be) but soft and clearly fully surviving, with no trace of a reaction. 7 show some hair-growth; 2 pretty good hair-growth; and 4 have almost complete pelts.

Re-inject each mouse i/p with 2ml Trypan Blue as used before.

[13] In general the grafts are fully pelted but some show apparent signs of weakness on clipping and poking at surface.

Pot 1: 5 x OK, 1 x ?weak, 1 x definitely weak.
Pot 2: 4 x OK (one cut into by mistake:Eo), 2 x ? weak, 1 x weak.

[14] Exact scoring is impossible; but most grafts are surely breaking down, leaving two or three genuine 100s.

[16] Situation is clarifying a little. Three grafts show further hair growth & supple dry well-pelted surfaces (in one, a really bushy & impressive growth). Clip these. Two show breakdown (possibly 3: kill the certain 2). The remainder have no further hair growth and have partially matted and scaly surfaces. These are partial survivals.

Pot 1: 75 50 50 25 25 0 + 100.
Pot 2: 25 25 25 0 + 2 x 100. 11/13 surviving.

[17] Less detailed inspection gives:- [H = high, L = low].

Pot 1: H L L L L 0 0 9/13 surviving, but only 3 with high
Pot 2: H H L L 0 0 degree of survival.

[18] 5 x 0: these grafts had matted carboardy surfaces, and breakdown may have been complete a day or two ago. 1 x 25 clearly. 3 x 100, grafts which though somewhat mushroomed have dry furry surfaces (hair has regrown since the last clipping) and firm blue surfaces of epithelium. 1/3 has a dorsal re-entrant showing weakness. Survivors 4/13; probably safest to take MST as 17 days.

(20) 1 x 0 contracted and hard
2 x 100- grafts rather thick and oedematous; one beginning to show signs of hardening
1 x 100 supple and without sign of reaction

(21) As above, but hardening is continuing in one graft. Still definite survival though. Examine carefully to-morrow. Reaction probably well under way in 2/3.

[22] Scores 100 (a perfect, supple graft with copious new hair growth & no sign of reaction. The texture and hair is still infantile)
75 clear partial survival
tr only.

-- Inject the 2 higher survivors with 2.0 ml trypan blue i/p. [Fourth injection: the previous 3 were done at days -1, +1, +11].

[23] 100 75 0 (kill). There is just a trace of hardening on dorsal rim of the 100: otherwise this graft is perfect; and the 75 is also holding up well.

[25] 100 75

[27] 100 75(or less)

[29] 100 but a central defect developing; note new hairs in past 4 days
75- No new hairs over clipped part, but more than 50% surviving.

[30] = Thu 11 Feb. Both grafts on way out, but both still surviving.

[31] The graft hitherto 100 now trace only - weak peeling surface. The other holding out at 50-.

[32] 0 (might be trace): the former 100. 25.
[33] 0/0.

SUMMARY: Injections of trypan blue [0.15ml 0.01M] on -1, +1, +11, +22.

LIFE TABLE [13 grafts]

Days:	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	31	32	33
Survivors:	13	11	9	4	3	3	3	3	2	2	2	2	2	2	2	2	1	0

MST: 17½ days MEL: 19¼ days

Trypan blue prolongs survival (contrast HYB-4 control: 9/9 grafts gone by 16 days certainly; perhaps by 14 days).

Newborn hybrid grafts, as usual, inferior in survival to adult hybrid grafts (see HYB-8 where in spite of one less trypan blue injection the MST & MEL were respectively 23 and 22-24 days).

ADULT CBA/A HYBRID GRAFTS ON CBA & A TREATED WITH ANTISERUM HYB-9

[First trial. Antiserum, see ANT-395, consisting of A & CBA mice respectively which have had six injections of 25 mg wet weight CBA/A hybrid lymphoid tissue. This is referred to below as "hyperimmune" serum.]

15 JAN 1960 Bleed 3 hyperimmune CBA-ff and 6 ditto A-mm for 1.5 and 2.8 ml serum respectively.

Inject 5 virgin CBA-ff with 0.30ml CBA antiserum s/c and 10 A-mm with 0.28 ml A-line antiserum s/c. These antisera should contain antibodies against the 'foreign' component of the hybrid grafts to be transplanted tomorrow.

16 JAN 1960 Test graft the 5 CBAs and 10 As with CBA/A hybrid skin. [Note that these CBA females came from a suspected stockpot in which there had been several deaths.]

See ANT-395 for hyperimmunization schedule.

18 JAN 1960 Bleed the 2 CBA & 4 A-line mice of ANT-395 hyperimmune series which did not get a booster dose on 16 Jan [see ANT-395]. These mice thus have thus had 6 injections of which the 6th was 14 days ago exactly.

Inject the 5 CBAs with 0.2ml CBA-antiserum s/c each, and the 10 As with 0.23ml s/c each of A-antiserum. Total dosages now: CBAs, 0.5ml; As, 0.51ml.

20 JAN 1960 Bleed 2 CBAs and 4 As from the ANT-395 hyperimmunization group (these mice & those used 23 Jan received a booster dose 16 Jan). Dilute

the respective antisera to 5.0ml with normal saline. Inject the 5CBAs with net dose of 0.22ml CBA antiserum s/c and the 10 As with 0.24ml A antiserum s/c.

[The CBAs look scraggy & rather wasted:- special diet.]

23 JAN 1960 Bleed 2 CBA and 4 A hyperimmune mice and prepare sera. Dilute the CBA serum to 5.0ml and the A serum to 10.0ml with normal saline. Inject each mouse s/c with 1.0 ml. Dose - CBA = 0.28ml neat serum
A = 0.14ml " "
A serum in short supply because 1 mouse died before bleeding.
Note that the mice are in much better shape.

25 JAN 1960 [9] INSPECTION. The 10+5 grafts well healed: all are thin and rather unexpedtedly delicate-looking, but all have 100% epithelial cover which stood up after an hour's exposure. Follow.

[10] A poor lot of grafts. CBAs: 4 x 90. One graft was gnawed off & is represented only by a tag. Kill. As: No mrk pot: 100pig/100/100-/75/50. EoH mark: 100/100/100-/100-/90. All grafts look fragile, with some serous exudation: the appearance is of transparency and breakdown seems to be taking the form of a fading away of epidermis. Not unlike a low-dose cortisone picture.

Reinjection: Bleed the last CBA and last 2 As from ANT-395; add one CBA and 2 As from HYB-11. Inject CBAs (4 only) with 0.29ml CBA antiserum and 10 As with 0.2ml A antiserum.

[12] The grafts continue poor. Many of those of As show pigment shadows.

CBAs - 4 x 75/90. Grafts contracted and rather scaly.
As - EoH. 100-/100-/90/90/75
No mrk. 100-/90/75/50/25 (this last the chewed graft).

[13] CBAs - 25/25/25/0 & not worth reinjecting
29 Jan As - EoH. 100-h/tr/tr/0/0 As: 7/10 survivors
No mrk. 100-h/90h/50/tr/0
The hairs are barely pierced, but there is an underlying dense pigmentation.

Bleed 3 As of HYB-11 q.v. Get 1.4ml serum, dilute to 3.0 ml, and inject the three high-surviving A grafts only with 1.0ml = 0.45ml immune serum each.

[14] CBAs - 25/25/0/0
As - 100-/100-/90/25/0/0/0/0/0/0 = 4/10 survivors. No further progress of hairs. MST 13½ days.

(16) CBAs - 0/0/0/0
As - 100-/75/0/0 Note a few feeble but pigmented hairs on the 100-

(17) As - 75/50 Reinject s/c each survivor with 0.28 ml of neat anti-serum obtained from one HYB-11 mouse.

[18] Scores 25 & (kill) tr. Thus even with breakdown in progress the immune serum has failed to deliver a coup de grace.

[19] 25 & 0

[20] 0

SUMMARY (CBA→A only)

Injections of hyperimmune serum & no. of surviving grafts:

day	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
grafts		10	.	.	.	.	.	.	.	.	.	10	10	10	7	4	3	2	2	2	1	0
ml a/s		.28			.23		.24			.14		.20			.45				.28			

Total antiserum: 1.82 ml

MST = 13½ days MEL = 14.1 days

Controls: See summary of HYB-7. Taking the pooled data there is a one-day advance in MST, and 2/10 survive 18 days instead of 1/32. Marginal significance, but the effect, <u>if any</u>, is a slight enhancement.

Compare also HYB-7 alone with the above:-

	HYB-7 (20)	HYB-9 (10)
MST	12+	13+
MEL	11.8	14.1

ADULT CBA/A HYBRID GRAFTS ON MICE TREATED WITH TRYPAN BLUE HYB-8

[Strict controls: HYB-7; but see also HYB-3,5. This expt repeats HYB-6 using A-line hosts only and giving same initial dose (i.e. 0.6ml 0.01M trypan blue administered 2 days before & two days after grafting. Booster doses will be given to some at least.]

30 NOV 1959 Warm a 0.01M solution of trypan blue (details, AN T-390 for details of sample) in 0.10M phosphate buffer pH 6.9 approx. Dilute 15ml to 100ml with normal saline. 2ml now equivalent to the standard "half dose" i.e. equivalent to 0.3ml stock solution 0.01M.

Now inject 20 large A-mm with 2.0 ml i/p each. No leaks. To be repeated 2 days after grafting.

2 DEC 1959 The 20 injected mice vary very much in degree of blueness. Give all the standard or -1 dose of nembutal, & graft with CBAm/Af hybrid skin (no sex-linked complications as recipients aremales).

4 DEC 1959 Reinject all 20 mice with 2.0ml trypan blue (sample homogeneous with that used 30 Nov., ≡ 0.3ml 0.01 M dye).

12 DEC 1959 [10] 20 x 100 clearly.

[11] Most grafts have central ?scabs: scores are low, but will probably improve for several days when grafts settle down.

[11] 4 x 100, 6 x 90, 9 x 75, 1 x 25 [but not a genuine breakdown: part of this 25 graft simple eaten away: rest normal].

[12] 5 x 100, 8 x 90, 5 x 75, 2 x 25 (of which one is probably genuine).

-- REINJECT all mice i/p with ≡ 0.3ml 0.01M trypan blue (2ml of solution made by diluting 15ml 0.01M trypan blue to 100ml with 0.15M saline). --

[13] Mice OK: the largest would probably take another injection in a few days. Essentially no change in the grafts: 5 x 100, 8 x 90, 4 x 75, 1 x 50, 2 x 25 (one the original gnawed one; the other has also been chewed up, but probably has a dorsal 25% intact).

[14] 3 x 100, 5 x 90, 7 x 75, 3 x 50, 25 (the original), 0 (probably not entirely genuine). 19/20

These scores suggest slow deterioration; but there is no doubt that the trypan blue is interfering seriously with healing, and thus preventing the normal healing process of the tiny ulcers on most of the grafts. These may not represent true breakdowns at all.

[15] 4 x 100-h; 2 x 90h; 4 x 75h; 2 x 75; 4 x 50; 3 x 25; 0. Hair growth just beginning. 19/20

[16] 2 x 100-h; 3 x 90h; 4 x 75h; 3 x 50h; 25h; 4 x 25; 2 x tr (one = old 25). 18/20

[17] 2 x 100-, 4 x 90, 75, 4 x 50, 4 x 25, tr, 0 = 16/20.

[18] 4 x 90, 4 x 75, 7 x 25. Some further hair growth. 15/20 but going fast.

[19] 3 x 90, 3 x 75, 3 x 50, 3 x 25 = 12/20.

[20] 3 x 90, 4 x 75, 1 x 50, 4 x 25 = 12/20, essentially no change.

[21] 100-, 5 x 90, 2 x 50, 2 x 25 = 10/20. The "improvement" is due to the healing over of old scars; the grafts with higher scores are all more or less contracted, but are sound within their own limits. Little further hair growth.

[22] Signs of slight further hair growth: there has been a temporary improvement all round. The L00's indicate a graft complete within it original boundaries in spite of contracture.

4 x 100-cch+; 90h+, 90; 2 x 50h+; 50; 25 = 10/20.

[23] Some of the better grafts now going. The one 100 is on a very weak & emaciated mouse (has been so for a week).

2 x 100-, 3 x 90, 50, 3 x 25, tr = 10/20.

[24] 3 x 100-, 2 x 75, 3 x 25 = 8/10.

[25] 4 x 100- (little if any addition to very sparse & stunted hairs; much contraction; but grafts OK within own boundaries); 2 x 25, 2 x tr = 8/10.

[26] Pot 1/4. 50 contracted. Active patch may grow hairs.
Pot 2/4. Clip T - 100, well defined but hairs sparse & wispy
Clip H - 100, as Clip T, but hardening: will soon go?
No mark - 100 (the emaciated mouse). Almost no hairs.

Pot 3/4 - 0

[PTO

Pot 4/4 Clip T - trace
No mark - 100 (much contracted & odd looking, but new hairs have sprouted since yesterday)
0

Total 6/20survivors.

(27) Pot 1/4 50/25 (definite hair-growth, but appears to be contracting)
Pot 2/4 Clip T - 100 (definite hair-growth; looks pretty stable)
Clip H - 90 (definite hair-growth; hardening not marked; but elongated if very fine marginal scab; contracture?)
No mark - 100 (mouse rather sick and emaciated; no sign of hairs)
Pot 4/4 Clip T - 0 (kill)
No mark 100 (with hair-growth in full swing)

(28) Much as above, with continued hair-growth in some; but the last survivor of Pot 1/4 seems to be on way out. However, still some survival.

(29) Pot 1/4 - 0 or trace
Pot 2/4 - Clip T -- 100 (long wispy hairs, no scabs of any kind; stable?)
Clip H -- 90 (scab healing? Contracture)
No mark - 100 (still no hairs; animal still emaciated)
Pot 4/4 100 (best hair-growth seen in any, but even so rather patchy)

(30) Pot 1/4 0 (kill)

HYB-8]
Pot 2/4 Clip T -- 100 (unchanged)
Clip H -- 75 (new scab has appeared, and contracture continuing; still some hairs)
No mark - 100 (unchanged)
Pot 4/4 100 (unchanged)

(31) Pot 2/4 Clip T -- 100 (appears to be stable; graft not very large, as if contr. had once occurred; wispy hairs)
Clip H -- 50+ (scab enlarging; some wispy hairs remain; graft going)
No mark - 100- (still no hairs; very slight scabs; mouse still emaciated)
Pot 4/4 100 (good bunch of long hairs but not over whole graft surface; tiny "healed" scab ventrally)

Total 4/20 survivors

[32] Pot 2/4. Clip T — 100. Wispy hairs of poor colour (some straw-coloured) but graft well defined with correct dermal pattern. Graft has <u>improved</u> since PBM's last inspection [26] but is still far from perfect.
Clip H — 50. Holding on, but will presumably go in a few days.
No mark — 100? Nohairs; there are small blemishes on this graft that may mark beginning of end. (Note no hairs have regrown on surrounding clipped field. Emaciated mouse.)

Pot 4/4. 100. Has also improved slightly since last inspection [29] & hairs have come up on previously bald areas. <u>But</u> (a) tiny ventral scab; (b) dermal quality bad - white, fibrous; (c) some further contraction since [26]. Yet hair <u>colour</u> good.
Survivors 4/20 as before.

[33] Pot 2/4. Clip H - tr
Clip T - tiny central scab or blemish? Otherwise as before.
No mrk - no change.
Pot 4/4. 100 still. Scabs no worse, but maybe some more contraction.

[34] The general picture is one of extremely slow and progressive deterioration. Clip H in Pot 2 has now probably just gone: kill. Clip T: scab or blemish perceptibly larger. No mark - no change at 100. No hairs on graft or field; blemishes seem to have healed. Pot 4: some small scabs on anterior margin now. 3/20 survivors at 34 days.

[35] Pot 2: clip T now 25, rather rapid contraction
No mrk 100, but signs of weakness: hair follicles but no hairs
Pot 4: Further contraction: perhaps 1/5th original size. But hairs still well formed, & some may have grown in past few days.

[36] Pot 2: 0 and 100 (in latter there has been regn of ep over a small picked off area. Pigmented follicles but no pierced hairs.
Pot 4: 25: may be 0 tomoroow. 2/20 survivors

[37] Only one survivor, the 'emaciated' mouse: no change. 1/20 survivors

Autopsy of other two. Differed much in blueness. No macroscopic abnormality. Livers, lungs OK, ditto kidneys (very blue). Nodes might be a shade under normal weight; spleens (mean 152 mg) slightly enlarged. Adrenals normal.

[38] One surviving graft (emaciated mouse, still thin & shaky) has contracted slightly but is still complete within own limits. Dorsal half has definite pigmentary shadowing, but still no pierced hairs: will they come through before breakdown?

[40] 100-. Some further contracture, and pigmentation clear; otherwise no change.

[42] Slight further contracture.

[44] Ditto: signs of weakness of epidermis but still clear survival.

[45] Survival now clearly 25-50: VA region of graft gone.

[46] No change. 25-50% graft still solid; rest scabbed.

[47] The mouse has improved enormously, & suddenyl putting on weight; the surviving 50% of the graft now looks solid and supple, instead of thin and tissue papery: if it lasts, it might grow hairs.

[49] The improvement is sustained, and the graft seems to be repairing itself (50 score). Although there is some pigmentation, there are no hairs; if hairs fail altogether to form, it will become increasingly difficult to exclude overgrowth and progressive replacement. As it is, it may be that theapparent epithelial surface on the ventral tongue of the graft is overgrowth; but there is no reason to think that the posterior half of the graft is anything but foreign in origin.

[49 = 20th January]

(51) B.d. of very recent standing, possibly even trace survival. The epithelium peels off leaving a moist surface.
Note that the b.d. of the graft is fairly well correlated with the recovery of the mouse

(52) Confirm that b.d. is complete.

SUMMARY

CBA/A-A given 0.3ml 0.01M trypan blue at -2, +2, +12 days. These data cannot be pooled with those from mice which received only 2 doses.

20 mice: Life Table

12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	51	52
20	19	19	19	18	16	15	12	12	10	10	10	8	8	6	5	5	5	4	4	4	4	3	3	2	1	1	0

Note that the longest-term survivor (51 days) was a thin & emaciated mouse.

MST = 23 days

MEL = 23.8 days (including the emaciated mouse with 51 days survival)
22.4 days (excluding ditto).

[NOTE: These graftings are controls for HYB-8 q.v. They repeat HYB-3 & HYB-5 q.v., using A-line recipients only.

These controls are to be used for (a) SEN expeiments (immune mouse node cells→ guinea pigs) and (b) for ANT expts (effect of skin-graft pre-immunization on response of mice to trypan blue)]

1 DEC 1959 Graft 20 A-mm with CBAm/Af hybrid skin.

11 DEC 1959 [9] In spite of small size of grafts, healing is fair. Scores very clear: 19 x 100 or 100-; 1 x trace. Contrast HYB-5.

FOLLOW UP:

10 days: 11 x 100, 5 x 90, 2 x 75, 1 x 25, 2 x 0 = 18/20 survivors.

11 days: 3 x 100-, 4 x 75, 4 x 50, 4 x 25, 5 x 0 = 15/20.

12 days: 2 x 100, 90, 2 x 75, 3 x 50, 2 x 25, tr, 9 x 0 = 11/20.

13 days: 90, 75, 3 x 50, 15 x 0 = 5/20 survivors.

14 days: 100-, 50(? o/g), tr, 17 x 0 = 3/20 survivors. Note improvement of one graft.

[Separate 10 x 0 to use as ANT-396A q.v.]

15 days: 100, 19 x 0 = 1/20 survivors. The 100 has no hairs or clear pigment shadowing.

16 days: no change. The 100 may have contracted. No hairs or clear pigmet shadowing. Bold dermal pattern.

[17] The "100" has undergone contraction but is OK within its margins: it is just possible to discern a pigment shadowing.

[18] 50. The original long-survivor is now bunching up and going.

[19] 0 - expt complete.

SUMMARY [CBA/A→A only] [see HYB-5]

	9	10	11	12	13	14	15	16	17	18	19	20 days
Present expt	20	18	15	11	5	3	1	1	1	1	0	0
HYB-3,5	12	(10)	(9)	7	6	3	1	0	0	0	0	0
Total	32	(28)	(24)	18	11	6	2	1	1	1	0	0

MST about 12½ days

MEL about 11.9 days

HYB-7 only: MST = a shade over 12 days; MEL 11.80 days

ADULT CBA/A HYBRID GRAFTS ON MICE TREATED WITH TRYPAN BLUE HYB-6

[For controls, see HYB-3 & HYB-5. For Trypan Blue, see ANT-391 & ANT-392. In view of results of ANT-392, it was decided in this experiment to give the mice a "half dose" (0.3ml 0.01M trypan blue) 2 days before grafting and another half dose a few days after grafting.][Strict controls HYB-5.]

29 OCT 1959 Warm a stock solution of 0.01M trypan blue in 0.10M phosphate buffer at pH 7.0. Dilute 15.0 ml to 100 ml with normal saline. 2ml of this solution represents 0.3 ml of the original 0.01M trypan blue, i.e. is a half-dose.

Inject 13 CBAmm and 12 Amm with 2.0 ml i/p each.

31 OCT 1959 Graft CBA/A male hybrid skin upon the above (A's getting -0.3 ml weight-dose, CBA's getting - 0.5ml weight dose).

1 NOV 1959 Inject each mouse with 2.0 ml of a trypan blue solution homogeneous with that used on 29 Oct. The A's are fine; some of the CBA's are small and will die. Dose/mouse (as before) ≡ 0.3ml 0.01M trypan blue.

One CBA died later, leaving 12.

11 NOV 1959 [11] With one minor exception, all grafts perfectly healed. The mice are still bright blue. Controls: see HYB-5.

A-line hosts	CBA hosts
11 x 100 and perfect grafts. 1 x trace	11 x 100, 1 x ?75. Somewhat haemorrhagic and with adherent cuticles.

[12] Rapid deterioration of many grafts, giving picture inferior to single high dosage (0.6ml 0.01M trypan blue) controls (see ANT-392 summary).

A-line hosts	CBA hosts
100 100 100 100 100 90 90 75 50 25 0 0	100 100 100 100 100 100 90 75 75 50 25 25
13 days	
100 100 100 100 100 90 75 75 25c 25 0 0	100 100 100 100 100 100 90 75 75 25 25 10
14 days	
100 100 100 100 100 90 75 50 25 25 0 0	100 100 100 100 100 90cc 75 75 75 50 25 tr

15 days

100h 100h 100h 100-h 100-h 75h
50 25 25 25 0 0

100h 100-s 100-s 100-s 100-s 100-s
90 50 50 50 25 0

[h = clear hair growth; s = pigmentation shadowing]

16 days

100hh* 100hh* 100h 90h 75h 75h
50h 50 5 0 0 0

100h* 100h 100 100- 100-h 100-
90 75 50 25 25 0

*grafts of super appearance.

17 days

100hh* 100hh* 75h 75h 75h 75h
50h 25 0 0 0 0

100h* 100h 100h 100h 100s+ 90h++
75 75- 25 10 5 0

Further hair growth on the two supergrafts; rest deteriorating slowly.

CBA's pulling ahead? + = swollen; some pigmentation; ++ = emaciated animal: kill. This graft deteriorating anyway

18 days

100*h+ 100-h+75-h+ 75h+
50-h 25h 25 0 0 0 0 0

100*h+ 100*h+ 100h 100(h) swollen 100h
50 50 2510 0 0 0

Symbol+ = further hair growth since yesterday. Note that this is happening in deteriorating grafts.

One of the *grafts may be less swollen than yesterday; one is still swollen with very backward hairs, but is hanging on successfully (note pigmentation).

	A-line hosts	CBA hosts
19 days	100h+ generalized hair growth 100-h+ palpable 75h+] These two grafts "entire" 75h+] & contracted: more hairs. 25 going: hairs already gone 10 trace only. 0 0 0 0 0 0	100h+ more hairs: super graft 100h+ 100h+ scrappy hairs since last time 100 swollen: still pigmentation & very fine downy hairs 50 50 25 0 0 0 0 [note one death]
20 days	100h+ ?just palpable 90h+ but blemishes, some contrctn Two grafts about 50% original area but now apparently stabilized within new boundary & with additional patchy hair growth. 10 10 0 0 0 0 0 0	100-h+ with central blemish: new hairs but slow growth: some reaction. Three grafts apparently stabilized as under A notes, but at a 90% rather than 50% level; patchy new hairs. 75 50 25 25- 0 0 0 0
21 days	100h: no further hair growth, but still looks perfect. 75:- going: was one of the super grafts. 75. Probably going. One apparently "stabilized" graft, much contracted. 0 0 0 0 0 0 0 0	100-h central blemish slightly larger: no further hairs. 100-h Thickened graft 100-h Stabilized? sparse hairs 90h Sparse scappy hairs;? stable. 50 Bald 0 0 0 0 0 0 0

22 days: The super A graft has contracted slightly & is developing a scab.
One CBA graft is a 25-er

	A-line hosts	CBA-hosts
TWENTY-THREE DAYS	90. Super-graft now somewhat contracted, hardened; one plaque-like scab; but ? active areas. Stabilized graft with some surviving ep and ? active hairs. 25 Definite dorsal patch of surviving epidermis. ??0 But deep dermis might be surviving and active. 0 0 0 0 0 0 0 0 0	75 Hard & domed but possible active 75 Alaos with active-looking patches 50 Clearly surviving 10 0 0 0 0 0 0 (0)
24 DAYS	Former super-graft now 25, almost certainly going. Stabilized graft with solid surface and ? new hair growth. 25 25 0 0 0 0 0 0 0 0	25 ?Stabilized graft, but scabby still Trace 0 0 0 0 0 0 0 0
25 days	One survivor: the "stabilized" graft referred to above which now shows central weakness.	One survivor: the "stabilized" graft, now scoring 50 approx & looking as if it were going.
26 days	Some improvement: small, but still supple & well defined	A poor graft, obviously struggling; but still some survival.

27 DAYS [NOV 27]. Both the surviving grafts have now hardened over, and unless a recovery occurs should be scored tr today & 0 thereafter.
→→ Grafts followed to 9th December showed no recovery.

SUMMARY TABLE: HYBRID GRAFTS [controls HYB-5; see also HYB-3] on MICE RECEIVING 0.6ml 0.01M trypan blue (equally subdivided into one injection 2 days before and one one day after grafting).

	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28 days
CBA/A→ A	12	10	10	10	10	9	8	7	6	6	4	4	4	4	1	1	1	0
CBA/A→ CBA	12	12	12	12	11	11	11	9	8	7	5	5	4	3	1	1	1	0

CBA/A→ A	M.S.T. = 20	Mean expectation of life = 18.9 days		
CBA/A→ CBA	- 20.5	-	- - -	20.4 -

Contrast also with the 0.6ml (high) dosage figures for non- hybrid grafts:- [see ANT-394]

CBA→ A	M.S.T. = 20.5	M.E.L. = 20.3 days
A→CBA	- 21.0	- 20.3

Thus there is n.s.d. between hybrid and parental grafts; but hair growth etc was much superior on the animals receiving hybrid grafts; and the straight control (HYB-5) showed an MST barely different from parental grafts.

ADULT CBA/A HYBRID GRAFTS ON CBA & A-LINE MICE HYB-5

[Repetition of HYB-3 q.v.] [Strict controls for HYB-6 q.v.]

31 OCT 1959 Graft CBA/A male hybrid grafts upon 6 CBA males and 6 A males.

11 NOV 1959 [11] All grafts perfectly healed and results perfectly clear.

A/CBA → A	100 0 0 0 0 0		A/CBA → CBA	100- 10 0 0 0 0
[12] A/CBA → A	Trace 0 0 0 0 0		A/CBA → CBA	90 0 0 0 0 0
[13]	0 0 0 0 0 0 completes			75 0 0 0 0 0
[14]				10 0 0 0 0 0
[15]				0 0 0 0 0 0 completes

PTO FOR SUMMARIES

SUMMARY OF HYB-3 & HYB-5. Note: the results are put in separately & as totals, because it seems doubtful if pooling is justified.

		9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
CBA/A→CBA	HYB-3	6	6	6	6	4	3	3	3	1	1	1	1	1	1	1	1	1	1
	HYB-5	(6)	(6)	2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
	Total	(12	12)	8	7	5	4	3	3	1	1	1	1	1	1	1	1	1	1
CBA/A→ A	HYB-3	6	6	6	6	5	3	1	0	0	0	0	0	0	0	0	0	0	0
	HYB-5	(6)	(?)	(?)	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total	12	?	?	7	6	3	1	0	0	0	0	0	0	0	0	0	0	0

MEL: from pooled data, MEL CBA/A→ CBA is 12½ days
CBA/A→ A is 13 days

TRANSPLANTATION OF NEWBORN CBA/A HYBRID SKIN TO ADULT ~~CBA~~ A MICE HYB-4

16 AUG 1959 Litter of 9 born of CBAm x Af parents, timed mating. Remove 1 large graft from back and sides of each mouse and carefully scrape off the panniculus. Grafts beautifully thin.

Graft to 9 adult male ~~CBA~~ A mice. RHS.

17 AUG 1959 [11] Perfect healing: 9 x 100, the grafts being fantastically super in appearance, for (a) they have grown to the extent of causing a definite buckling, and (b) all are densely haired. Thus they are enormously large and black-looking. At present all are soft and possibly oedematous. There are slight indications that a few will give way in the next few days.

[Note on this expt. If 'adaptation' occurs it should be due to replacement of CBA by A antigens on surface of cells, and this may depend on the antigens' not having reached full expression in newborn skin. On the other hand, if they havn't reached full expression, they probably will not immunize the host in such a way as to provide the selective force driving CBA antigens off the surface. Should these grafts be put on to pre-immunized mice, or mice passively immunized with antiserum?]

12 days: 6 x 100; 2 x 100- (slight signs of going, viz: some contraction and induration); 1 x 0. This last graft was well contracted and rather hard; it came right off, easily, on poking. As this may be partly a self-undermining effect, keep animal & look out for pigmentation in (at present highly granulating) graft bed. The 6 100's are superb.

13 days. Grafts now becoming difficult to score. No further increase in size of hairiness; surviving grafts still oedmatous and would certainly show trenendoud round cell infiltrations. Apart from 0 already noted at 12 days, 3 or 4 more seem to be going (hardening, contracture, tendency for edge to disengage). Three or four grafts seem still O.K.

14 days. The apparent survival of these grafts (with one possible exception) can now be seen to be illusory. The grafts which look as if surviving, though slightly contracted and hardened and with non-water repellent surfaces, are in fact dead They peel off to reveal highly grabulating beds without bleeding.

15 days. Grafts have pretty well all gone, though keep for a short while in case pigmented hairs should sprout from apparently raw granulating tissue left after removal. The one graft that still looked surviving, with a dense pelt of hairs, is probably bogus: a strip was torn off to show a granulating bed with no bleeding whatsoever.

16 days. Subject to possibility that black hairs may sprout in graft bed, all grafts can now be taken as 0.

18 days. Kill.

HYB-3

CONTROL EXPMT: ADULT CBA/A HYBRID SKIN GRAFTS TRANSPLANTED TO A & CBA

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29 JUL 1959 Donor: male CBAm/Af hybrid. Grafts generally non-active though one or two have tiny active corners.

Recipeints: 6 CBA males
6 A males

8 AUG 1959 (10) A mice: 100/100-/90/75/50/25 but wth bad fault in healing
CBA " : 100/100/100-/75/75but infected/25but faulty healing

10 AUG 1959
12 days: A mice .... 100/75+/75/50-/50-25/25
CBA " .... 100/100/100-/75/25+/10

11 AUG 1959 (13) HYB-3A ... 2 x100,100-,90,25, trace.
HYB-3B ... 100-,90,3 x 50, trace.

12 AUG 1959 (14)
HYB-3A(CBA)... 1 x 100, 2 x 90-,50, 10, trace.
HYB-3B(A) ... 2 x 75, 50, 25-, 10, 0.

13 AUG 1959 (15) HYB-3A(CBA) ...100,2 x 90, 2 x trace, 0.
HYB-3B(A) ...75,10,2 x trace, 2 x 0.

14 AUG 1959 (16) A. (CBA)... 100,100-,50 ,10, trace,0
B. (A) ... 25-,10,4 x 0.

15 AUG 1959 (17) A. (CBA) ... 100,25, 4 x 0
B. (A) ... 6 x 0.

17 AUG 1959 [19] With one exception (see below) all grafts show t.b.l.s., but many show well developed marginal overgrowth pictures giving illusion of partial survival. Hence discount some of the "25" and "trace" scores above, which are probably not genuine. Kill all mice except the one noted below.

In 3A (CBA hosts) there is one perfectly surviving graft -- uncontracted, with a matt surface having the proper dermal grain and a well defined outline. The pattern of hair growth is as shown; note that there is a small central island of agouti hairs with pigmentation beneath. As this graft has been followed daily there can be no doubt at all about its genuine survival. Follow.

20 days Above graft OK, with further hair growth.

21 days No change

22 days No change: no further areas of graft have sprouted, but growth is fairly dense where present at all. Note exact graft-specific orientation of hairs.

23 days There not seem to be some wispy and almost colourless hairs over formerly bald part of graft. Rest of pelt is now more even. Otherwise no change.

24 days [= 22 Aug]. Perhas slight further hair growth. Quite a good-looking graft.

26 days. Mouse died sometime yesterday: it had been known to be off colour but cause of death is unknown. It is not wasted. Kidney & liver looked normal; nodes and spleens of right size.

The graft at 25 days is perfectly defined and uncontracted, with normal supple dermis and good "grain", and a hair pelt a bit whitish in late-growing areas, but of a good agouti colour where hair growth started. This would pass as a "fairly good" tolerated graft.

SUMMARY

If scores of 25 & below are discounted* for the reasons given above, the life tables become:-

days:-	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	
Hyb → CBA:-	6	4	4	3	3	1	1	1	1	1	1	1	1	1	1	(mouse died)
Hyb → A :-	6	5	3	1	0	0	0	0	0	0	0	0	0	0	0	

*not at 12 days, when scorings were done by L.B.

CBA

TRANSPLANTATION OF CBA/A HYBRID NEWBORN SKIN GRAFTS TO & STRAIN ADULTS HYB-2

16 JUL 1959 Donor mice: litter from CBAm/Af mating. Remove from the back of each one largish graft, trim by gentle scraping away of panniculus with scalpel blade. Sex of donors unknown, but note that recipients are males.

Recipients: 6 male adult CBA mice. RHS opns.

25 JUL 1959 (9) 6 x perfect grafts, with pigmentation and incipient hairgrowth

27 JUL 1959 (11) 1 x perfect, with rich and supple pelt of fine hairs
1 x 100, partly covered with pelt, uncontracted
1 x 100, ditto
1 x 100-, ditto but some contracture and light scabs
2 x 90, some contracture and scabs, only few hairs

29 JUL 1959 (12) Situation essentially unchanged: 3 good grafts, and 3 not so good ; the latter are probably going, but due to the thickness and intense activity of the grafts exact survival scores are of dubious value.

Later p.m. One graft probably with no survival or rather little. The other 2 bad grafts undoubtedly have partial survival

29 JUL 1959 (13) 2 x 0; 1 x 50?; 2 x 100- (small superficial scabs now visible)
1 x 100 (still with exuberant pelt of hairs

30 JUL 1959 (14) 2 x 0; 1 x trace or 0 (undermining in progress)
2 x 90 (more or less uncontracted, pigmented, partly haired, clearly with predominantly surviving epith, but with some scurfiness and a few light and tiny scabs
1 x 100- (fully pelted, thick graft; difficult to score with accuracy, but a tiny marginal scab apart mothing obviously wrong with it)

31 JUL 1959 (15) 3 x 0, 1 x tr
2 x 25/50 (the fully pelted graft is very much contrcated, hard and beginning to be undermined at the margins)

1 AUG 1959 (16) 4 x 0;
2 x slight or no survival, b.d. just complete

2 AUG 1959 (17) Confirm b.d. in all grafts

TRANSPLANTATION OF NEWBORN A SKIN TO ADULT CBA MICE (CONTROL) HYB-1

23 JUN 1959

Grafts: removed from 6 newborn (less than 12 hours old) A strain mice, 2 males and 4 females. Grafts roughly standard size and comprising skin from the back and sides. Trim away, as far as possible, some of the subcutaneous fascia.

Recipients: 6 male CBA mice. RHS grafts. Mark the recipients of male skin with right ear notch.

2 JUL 1959 (9) INSPECTION: all grafts have healed beautifully, though one (mark mouse with tail clip) has a small marginal fault. Healthy pink and epithelialised, but noticeably swollen. In general, no sign of incipient breakdown, but one graft shows obvious scratch mark a few minutes after removal of bandage; this suggests epithelial weakness.

2 HOURS LATER: several of the grafts are really enormously swollen - domed. At least 4 of them have tiny patches of white downy hairs. 1 (the badly healed = tail clip) is contracting, and 1 is scabbing fast (only trace survival now). Scores: 100/100/100/100/90/tr.

3 JUL 1959 (10)
100 incip. hair growth, no contracture
100- ditto
100 incip. hair growth, but some contracture
75 incip. hair growth, but scabbing in progress
2 x 0

4 JUL 1959

(11) 75/50/50/25/0/0

(12) 25/25/0/0/0/0

(13) tr/ and 5 x 0

A number of blank pages follow
and have <u>not</u> been photographed.