rather than mixing of exogenous and endogenous cholesterol.

It would also appear that the cholesterol esters in these homogenates are inert. Labeled esters were not synthesized from cholesterol-4-C^{14} and when C^{14}-labeled esters were added to the homogenates, neither the free cholesterol nor the steroids produced during incubation were labeled. It is possible that the labeled esters did not exchange with the endogenous esters and become available as a source of labeled free cholesterol, or that the labeled esters used in this study are not utilized for steroid production although these are the major cholesterol esters of hog adrenal as determined by gas liquid chromatography.† Another possible explanation for this finding is that hog adrenal lacks cholesterol esterase since labeled esters added were not converted to free cholesterol. Thus, any labeled ester present, even if in an active pool, could not be converted to free cholesterol. The constant chemical levels of cholesterol esters in these homogenates lend support to this possibility.

Summary. Tracer amounts of cholesterol-4-C^{14} added to hog adrenal homogenates and incubated under conditions favorable for steroid synthesis were rapidly and efficiently converted to labeled steroids. Addition of cholesterol-4-C^{14} esters, prepared enzymatically and incubated under the same conditions, did not give C^{14}-activity in the steroids produced. Conversion of free cholesterol to cholesterol esters or of esters to free cholesterol as measured by C^{14}-activity of the sterol digitonides did not occur in this system.

† Unpublished findings.

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Received March 30, 1962. P.S.E.B.M., 1962, v110.

Measurement of Serum Binding Capacity for Vitamin B_{12}. (27583)

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Several investigators have dealt with the problems of binding of vit B_{12} by serum and serum protein fractions and how "free" and "bound" vit B_{12} are distributed in biological material(1-6).

The determinations of "bound" vit B_{12} have been carried out by different methods, which may account for the varying results obtained. To explain some of the apparent discrepancies we have compared 4 different methods for assaying serum binding of vit B_{12} : dialysis; availability of vit B_{12} for *Lactobacillus leichmannii* and for *Euglena gracilis;* and uptake of vit B_{12} by wild type *Escherichia coli.*

Material. Sera were collected aseptically from 24 healthy adults and stored at –20°C in vials containing 1 ml serum each. The vit B_{12} content of each serum was assayed by the *L. leichmannii* method(7). For the binding experiments mixtures were made up of 0.8 ml serum and 0.2 ml of a sterile solution of vit B_{12} in 0.9% saline, containing 10 mg KCN per liter. This mixture was incubated aseptically at 28°C for 18 hours before assay.

Methods. a. *Dialysis.* The preincubated

TABLE I. Serum Vit. B_{12} Binding. pg ($=10^{-12}$g) per ml in a mixture of 0.8 ml serum and 0.2 ml of a vitamin B_{12} solution containing 3000 pg vitamin B_{12}.

	a	b	c	d	e	f	g
	L. leichm.	*L. leichmannii*		*E. gracilis*		*E. coli*	Dialysis
		"Free"	"Bound"	"Free"	"Bound"	"Bound"	"Bound"
No.	Total B_{12}	B_{12}	B_{12}	B_{12}	B_{12}	B_{12}	B_{12}
			a − b		a − d		
1	3170	665	2505	2850	320	1380	1000
2	3170	640	2530	2350	820	900	990
3	3215	820	2395	3000	215	1210	1260
4	3240	865	2375	2500	740	910	960
5	3245	895	2350	2420	825	1250	1130
6	3245	1220	2025	2750	495	1120	850
7	3250	765	2485	2920	330	900	1160
8	3250	795	2455	2540	710	1080	1260
9	3260	755	2505	2800	460	1060	1370
10	3270	855	2415	2430	840	950	960
11	3280	955	2325	3010	270	—	900
12	3295	1165	2130	3020	275	830	880
13	3300	700	2600	2600	700	1050	1260
14	3300	935	2365	2520	780	1270	880
15	3300	890	2410	2480	820	880	—
16	3305	990	2315	—	—	800	1160
17	3310	930	2380	2650	660	770	1040
18	3320	935	2385	3020	300	1300	1090
19	3340	745	2595	2650	690	1020	1350
20	3340	930	2410	2740	600	1180	1130
21	3345	1000	2345	3100	245	1070	1080
22	3420	810	2610	2850	570	1400	1070
23	3510	750	2760	2780	730	980	1050
24	3560	780	2780	3050	510	1120	1350
Avg	*3302*	886	*2436*	2740	*561*	*1062*	*1095*

mixtures of serum and vit B_{12} were dialyzed in Visking bags against 40 volumes of sterile saline at room temperature in a rotating apparatus. The saline was changed 3 times at 3-hour intervals. The serum proteins were precipitated by autoclaving at pH 5, and non-dialyzable vit B_{12} was assayed in the supernatant by the *L. leichmannii* method(7).

b. *Availability for* L. leichmannii. The pre-incubated mixture of serum and vit B_{12} was diluted aseptically and added to tubes containing autoclaved basal medium and enough water to give a final volume of 2 ml per tube. The growth-promoting effect of the serum vit B_{12} mixture was then determined in the usual way. By this method the amount of available or "free" vit B_{12} was determined. The amount of non-available or "bound" vit B_{12} was calculated by deducting available vit B_{12} from total amount of vit B_{12} present in the mixture.

c. *Availability for* E. gracilis. The method of Hutner, Bach & Ross(8) was employed, the principle for determination of available and non-available vit B_{12} being the same as that described for *L. leichmannii.*

d. E. coli *method.* The fraction of "bound" vit B_{12} not taken up by wild type *E. coli* cells was determined according to Hoff-Jørgensen (9).

Results and discussion. In Table I the results of vit B_{12} binding determinations obtained on 24 sera with the 4 methods are given. For the experiments 0.8-ml samples of serum were mixed with 3000 pg (1 pg (picrogram) $= 10^{-12}$ g) vit B_{12} in 0.2 ml saline solution. The Table shows that the 4 methods give different but consistent results and further, that with none of the methods is there any correlation between binding of vit B_{12} and concentration of vit B_{12} originally present in the sera.

Fig. 1 and 2 illustrate the effect of total vit B_{12} concentration on serum vit B_{12} binding capacity.

Determinations with dialysis and uptake

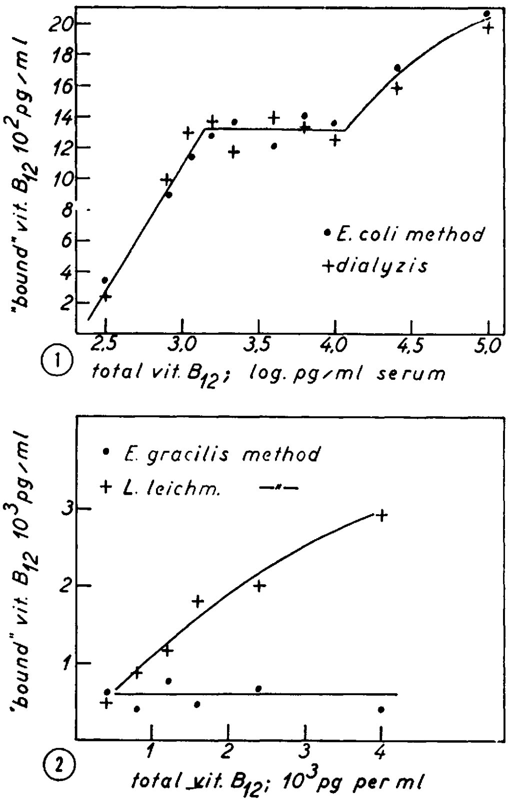

FIG. 1 and 2. Relationship between total and "bound" vit. B_{12} as determined by four methods. Each point represents the avg of 4 determinations on the same serum sample.

by wild type *E. coli* are in good agreement, while determinations with *L. leichmannii* and *E. gracilis* differ widely from these determinations and from each other.

The curves for *E. coli* and dialysis show a rise in the "bound" fraction of vit B_{12} to a total concentration of about 1600 pg/ml serum. With a total concentration from about 1600 pg/ml to about 10,000 pg/ml the binding is rather constant at about 1350 pg/ml and above this total concentration, "binding" again increases with increase in total concentration of vit B_{12}.

The determinations of binding capacity with *L. leichmannii* show far higher fractions of "bound" or non-available vit B_{12}, increasing almost proportionally with the increase in total vit B^{12} concentration.

Determinations with *E. gracilis* give much lower and constant values (600-700 pg/ml), which indicate that for *E. gracilis* unavailable vit B_{12} is independent of total concentration of vit B_{12}.

The results with the 2 latter methods indicate that the dialyzable vit B_{12}-fractions include some of the vit B_{12} unavailable for *L. leichmannii*, while *E. gracilis* is able to utilize some of the non-dialyzable vit B_{12}.

Summary. Four different methods for determination of "free" vit B_{12} and the fraction "bound" by the serum proteins have been compared. The serum binding capacity for vit B_{12} at varying concentrations of total vit B_{12} has been determined with the same methods. None of the methods showed correlation between original serum vit B_{12} concentrations and binding of added vit B_{12}. When binding capacity was determined by dialysis and by using uptake of vit B_{12} by wild type *E. coli*, there was good agreement. This showed a mean binding capacity of 1350 pg vit B_{12}/ml serum when total vit B_{12} concentration was below 10,000 pg/ml. Amount of vit B_{12} unavailable for *L. leichmannii* increased almost proportionally with total concentration of vit B_{12}. Amount of vit B_{12} unavailable for *E. gracilis* was almost constant, irrespective of total concentration of vit B_{12}.

We therefore propose that the term "microbiologically unavailable vit B^{12}" should always include the name of the organism and the initial total concentration of vit B_{12}, and that the serum binding capacity for vit B_{12} should be defined as the amount of vit B_{12} per ml serum which is non-dialyzable at such initial total concentration of vit B_{12} that more than one-fourth and less than half of the initial total amount of vit B_{12} remains undialyzed.

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Received April 9, 1962 P.S.E.B.M., 1962, v110.

H-Reflexes in Normal Human Infants: Depression of These Electrically Induced Reflexes (EIR's) in Sleep. (27584)

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In the normal human adult, weak percutaneous electrical stimulation of the tibial nerve excites low threshold calf muscle afferents which synapse directly on spinal motoneurons to evoke a reflex contraction from the same muscle group. This monosynaptic myotatic reflex, recorded by the action potentials evoked in the muscle, was extensively studied by Magladery *et al.*(1), who named it the H-reflex (after Paul Hoffmann who described it earlier). Thomas and Lambert(2) reported that the H-reflex could be easily elicited in the normal newborn infant by stimulating the ulnar nerve and recording the hypothenar muscles. Their finding that this electrically induced reflex* (EIR) could no longer be obtained in the normal infant over 6 months of age suggested to us its use as one attractive and simple test of the neurological status of the pediatric patient. The value of such testing is indicated in our recent demonstration of aberrant EIR's produced by ulnar nerve stimulation in children, 8 to 14 years old, with Sydenham's Chorea(3).

While using the ulnar EIR in a long-term serial study of growth of the normal infant from birth, as well as in an investigation of various neurological disorders, defective development, and mental retardation of the young patient, we have noted additional points of interest regarding the EIR in 13 normal neonates (1 to 8 days old), some of whom have been reexamined 2 to 4 months later.

Methods. Subjects were examined while lying undisturbed in the bassinet. Silver cup-shaped disc stimulating and recording electrodes, 6 mm in diameter, were applied to the skin with Bentonite paste and held in place by ¼-inch masking tape. One electrode for stimulating the ulnar nerve was a few cm distal to the axilla, the other at the olecranon groove of the elbow, interelectrode separation being 3.5 to 5 cm. Active recording electrodes were applied over the bellies of forearm flexor, hypothenar, and first dorsal interosseus muscles; indifferent electrodes for these muscle groups were located on the flexor surface at the wrist, and the tip of the fifth and index fingers, respectively. Routinely, square wave pulses of varying voltage, 0.2-0.5 millisecond (msec) in duration, were passed through an isolation transformer and delivered to the stimulating electrodes once per 2 seconds. Muscle action potentials were amplified by conventional biological amplifiers, displayed on a double beam cathode ray oscilloscope, and photographed on 35 mm moving film.

Results. Ulnar-hypothenar (UH) EIR's were easily obtained in all subjects, thus confirming Thomas and Lambert's(2) results. In addition, all subjects presented EIR's from forearm flexor (UFEIR) and dorsal interosseus (UIEIR) muscles upon stimulation of the ulnar nerve. Fig. 1 shows recordings of hypothenar (upper beam) and flexor (lower beam) muscles from a normal 6-day-old baby. These records demonstrate 2 main features

* This term, used hereafter, is more descriptive, of more general applicability, and less committed as regards mechanism and functional significance than the designation, H-reflex.