Album of anatomical drawings of human bones by Sir William Leishman

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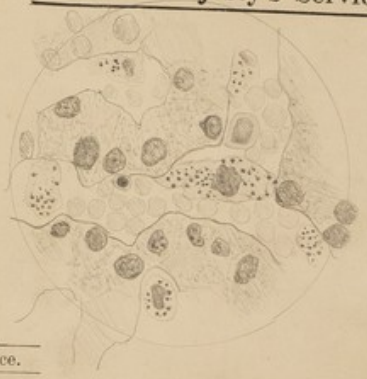

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2m obj. Oc 6. Zeiss apparat.
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'Carini' bodies from Lung of Rat
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June 27th 1914





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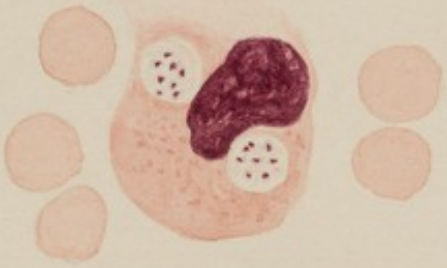

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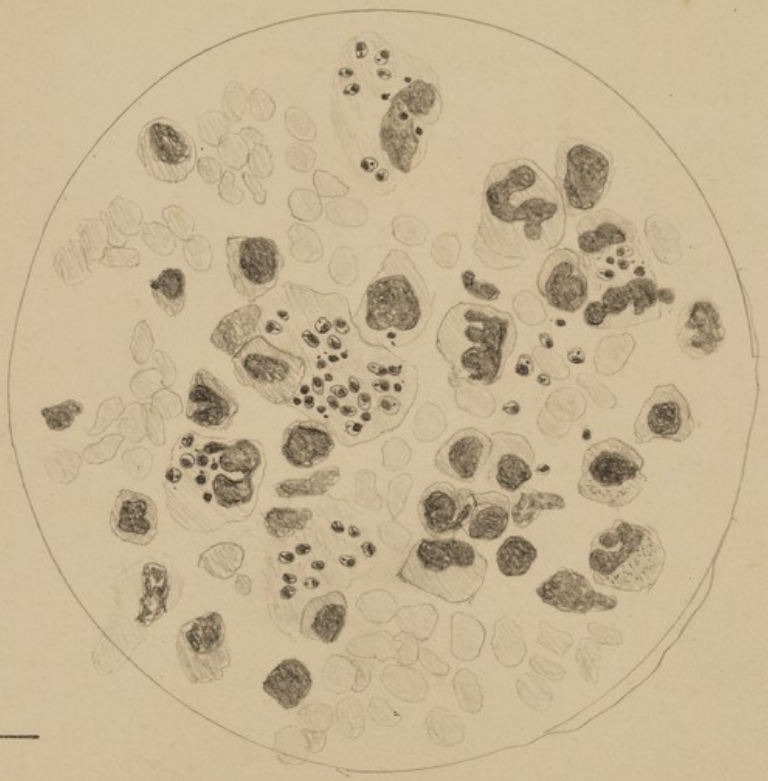

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Dott. A. TOMASELLI

STUDIO DELLE LEISHMANIE
nel succo della milza dei bambini affetti da kala-azar

Estratto dal Policlinico, *Volume XVII-M., 1910*

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Roma, 1910 — Tipografia Nazionale di G. Bertero & C.

Istituto di clinica medica della R. Università di Catania
diretto dal prof. R. Feletti

Studio delle Leishmanie nel succo della milza dei bambini affetti da kala-azar

per il dott. A. TOMASELLI, assistente.

(Con una tavola litografica a colori).

È mia intenzione di occuparmi in questa nota di alcune particolarità riguardanti la morfologia e lo sviluppo dei corpi di Leishman nella milza degli ammalati di kala-azar infantile. La ricerca dei parassiti in altri organi non ci è stata permessa in alcun modo.

L'argomento è stato oggetto di studio da parte di alcuni autori. La morfologia di tali corpi oltre che dallo stesso Leishman è stata anche descritta de Pianese, Nicolle, Gabbi, Feletti ed altri. E se nelle linee generali esiste una grande analogia tra i diversi reperti, tuttavia per alcuni dati l'accordo non è stato ancora raggiunto.

Il Leishman ha descritto un solo tipo di parassita costituito da una massa protoplasmatica ovale o rotondeggiante, jalina, a bordi netti, talora vacuolizzata e da due ammassi cromatinici, uno più grosso (nucleo), uno più piccolo, bastonciniforme (blefaroplasto). Lo stesso autore talvolta ha trovato anche forme con due grosse masse cromatiniche e con due nuclei nello stesso corpo; il che ha fatto pensare ad una moltiplicazione per semplice divisione.

Questi corpicciuoli si sono presentati ad ammassi od isolati; nel primo caso apparivano inclusi in una sostanza fondamentale colorantesi in bleu col Giemsa, con aspetto di protoplasma granulare.

Il Pianese ha distinto i corpi di Leishman in tre forme: una grande, ovalare o piriforme con membrana, protoplasma, nucleo e blefaroplasto; una

di media grandezza rotonda a membrana, protoplasma e nucleo; la terza di minima grandezza, rotondeggiante con nucleo e scarso alone protoplasmatico. Il protoplasma è descritto da lui come tenuissimo, finamente granuloso, mai vacuolare. Riguardo alla sede ammette che siano tutti intracellulari, talvolta raggruppati in cumoli che hanno intorno una listerella di protoplasma addensato che forma una specie di capsula. In quanto allo sviluppo il Pianese ha ritenuto che la forma piccola e di più semplice struttura rappresentasse la fase iniziale del ciclo evolutivo che il parassita percorrerà dentro la cellula della milza. Ed avendo egli notato che la forma ovalare — più evoluta — si riscontrava più nel midollo delle ossa che nella milza; la rotonda, di media grandezza, meno evoluta, più nel fegato che nella milza; e la rotonda di minima grandezza — poco evoluta e iniziale — soltanto nel fegato, ne deduceva che l'organo, nel quale il parassita prima si colonizza, fosse il midollo delle ossa, a cui avrebbe seguito la milza e poi il fegato, ed ulteriormente le ghiandole linfatiche e forse altri organi.

Riguardo alla moltiplicazione, nè in preparati a secco nè a fresco, ha potuto osservare alcun accenno a moltiplicazione.

Il Nicolle nei preparati per strisciamento del succo splenico ha visto i parassiti o liberi o intracellulari, ovvero riuniti in una specie di *ganga*. I corpi liberi erano costituiti da protoplasma e da due cariosomi; quelli intracellulari erano contenuti nelle grandi cellule mononucleari. Riguardo alla sostanza mal definibile, chiamata *ganga* da Laveran e Mesnil, il Nicolle ha ritenuto che fosse un pezzo del corpo dei leucociti e delle cellule della milza, che viene a staccarsi in seguito a strisciamento. Ha visto infine nel sangue splenico alcune forme di moltiplicazione del parassita, la quale avviene per divisione longitudinale.

Anche il Feletti, in una nota preventiva di recentissima pubblicazione, ha portato un contributo allo studio della morfologia e biologia dei corpi di Leishman nella milza.

Sullo stesso argomento rapidamente accennato dal mio Maestro, desidero intrattenermi.

I preparati che servirono allo studio, furono allestiti con succo della milza. Alcuni vennero fissati in alcool-etere od in alcool metilico e colorati col liquido di Giemsa; altri infine furono osservati a fresco senza colorazione alcuna. Un buon metodo per accertarsi della presenza o meno dei parassiti nel succo splenico mi è parso il seguente: 1° mettere per pochi minuti (10-15) i vetrini porta-oggetti sui quali è stato disteso il succo da esaminare, in alcool-etere; 2° porre una goccia di una soluzione acquosa di bleu di metilene su un vetrino copri-oggetto e sovrapporlo al vetrino porta-oggetto. Così facendo, dopo qualche minuto i parassiti ricercati, se sono presenti saranno ben colorati. In tal caso si leva il vetrino copri-oggetto, si asciuga l'altro, il quale rimane così utilizzabile.

Nel caso in cui la colorazione al bleu di metilene non riuscisse gradita, si scolorerà il vetrino porta-oggetti in alcool e si colorerà con la soluzione preferita.

Tale metodo più semplice, più rapido degli altri, mi è parso anche migliore di quello della colorazione vitale, poichè quest'ultimo non permette di utilizzare ulteriormente il preparato che è servito per la prima osservazione fatta a scopo diagnostico. Da uno studio sintetico dei reperti pazientemente raccolti, è stato possibile rilevare alcuni dati che andrò esponendo.

Nei miei preparati di succo splenico i corpi di Leishman si presentano sotto differenti aspetti che non è difficile corrispondano a varie fasi della vita del parassita.

Gli elementi per così dire primordiali sarebbero rappresentati da piccole forme il cui diametro longitudinale è di 1-1.5 μ e quello trasversale di 0.5-1 μ. In esse un grosso nucleo fa contrasto con lo scarso protoplasma. Il corpo protoplasmatico è di forma ovoidale. Il nucleo sempre eccentrico, sovente trovasi spinto verso l'estremità appuntita. Non si notano mai vacuoli nè dentro il nucleo, nè dentro il protoplasma di questi piccoli elementi. In essi il blefaroplasto non è visibile (fig. 1).

Esistono molte forme di media grandezza il cui diametro longitudinale è di 1.5-2 μ, e quello trasversale di 1-1.5 μ. La loro forma prevalentemente è ovoidale; il nucleo suole essere disposto perifericamente e medialmente. Esso appare finamente granuloso; il blefaroplasto è puntiforme (fig. 2).

Finalmente si notano corpi di maggior dimensione; il loro diametro longitudinale misura 2-2.5 μ; quello trasversale 1.5-2.5 μ. La loro figura è rotondeggiante od ovale; il nucleo è più grosso che nelle altre forme. Il blefaroplasto sovente è bastonciniforme; in alcuni però è puntiforme. Tale vario aspetto del blefaroplasto è probabile sia dovuto alla posizione del parassita sotto il campo del microscopio (fig. 3).

Alcune considerazioni possono farsi sui reperti già descritti. I nostri preparati anzitutto non ci autorizzano ad ammettere che le forme grandi siano sempre ovalari o piriformi e le medie e piccole costantemente rotonde. Abbiamo infatti osservato delle forme grandi rotondeggianti e delle piccole ovalari o piriformi. Nè ci si può obbiettare che ciò sia dovuto allo strisciamento poichè queste forme ci sono apparse anche tali nei preparati allestiti a fresco o con la colorazione vitale, in cui non ho usato strisciamento. Il fatto poi che ci fu possibile vedere nel succo della milza delle piccole forme costituite da un nucleo e protoplasma, ci fa ammettere che tali corpicciuoli non siano soltanto nel fegato (Pianese). Quindi ci sembra non abbastanza fondata l'ipotesi che essendo i parassiti più piccoli solo nel fegato, l'organo nel quale il parassita prima si colonizza sia il midollo delle ossa a cui seguirebbero la milza ed il fegato.

La moltiplicazione dei corpi di Leishman dentro la milza avviene per

un processo di divisione longitudinale. Il primo a dividersi è il nucleo; si vedono infatti alcuni parassiti in cui il nucleo si presenta falcato mentre il corpo cellulare non presenta accenno a divisione (fig. 4); in altri elementi il nucleo è diviso in due blocchetti cromatinici a margini ben distinti ed il corpo protoplasmatico non è ancora diviso (fig. 5). In stadî successivi anche il corpo si divide in due secondo l'asse longitudinale. Tale divisione abbiamo potuto seguirla nelle sue diverse fasi; s'inizia alle due estremità del corpo di Leishman con una incisura per parte (fig. 6); queste due incisure si fanno sempre più profonde in modo da dividere in due il parassita (fig. 7). Ciascuno dei due elementi neoformati si compone del protoplasma e del nucleo.

Difficile a spiegarsi è il comportamento del blefaroplasto. Questo corpicciuolo negli elementi in cui il nucleo è in divisione incipiente o completa, talvolta manca. Altre volte invece appare disposto trasversalmente tra i due nuclei, ovvero soltanto in uno dei due elementi in formazione. Una spiegazione sicura di questi reperti non è facile. Nè dai miei preparati ho potuto stabilire se avvenga anche una divisione del blefaroplasto oppure se questo si formi secondariamente dal nucleo dei parassiti già divisi.

La sede della moltiplicazione verosimilmente è il corpo delle grandi cellule della milza e dei grossi mononucleati. Infatti in essi ci è possibile vedere riuniti in quantità talora considerevole i parassiti di Leishman. Altre volte questi li abbiamo visti dentro una delle così dette ganghe. Riguardo al significato di queste ultime sembra verosimile la definizione che ne ha dato il Nicolle, cioè che esse siano un residuo del corpo delle cellule della milza.

Le ganghe (fig. 8-9) con la soluzione del Giemsa, generalmente assumono un colorito roseo; raramente unc bleuastro. Esse si presentano rotondeggianti, di aspetto ialino, di varia dimensione e contengono un numero più o meno ricco di parassiti. Questi ultimi in maggior parte sono piccoli; di alcuni si vede solo il nucleo; di altri si vede anche il blefaroplasto puntiforme molto avvicinato al nucleo. Si vedono inoltre dei corpi in cui è ben disegnato anche il margine protoplasmatico ed in qualcuno di essi il nucleo appare in incipiente divisione (fig. 8-*b*). Nella figura 9 si vede una ganga che ha l'aspetto di un nido da cui stanno per liberarsi dei piccoli parassiti. Essi pare che in questa abbiano trovato culla ed alimento.

Accanto alle forme già descritte nella loro differente fase di sviluppo, ne vanno ancora ricordate altre che per i loro caratteri rappresenterebbero una fase involutiva. Questa s'inizia con una vauolizzazione del corpo protoplasmatico (fig. 10) il quale appare rigonfiato, a margini meno netti, colorato in bleuastro con la soluzione del Giemsa. Anche il nucleo dopo il protoplasma subisce il fenomeno della vacuolizzazione e quindi lentamente si dissolve (fig. 11). Pare che per ultimo resti il bleforoplasto (fig. 12) il quale anch'esso è destinato a scomparire benchè si mantenga ben colorato anche quando il processo di degenerazione vacuolare ha invaso il corpo cellulare ed il nucleo.

Conclusioni.

Riassumendo, dalla morfologia dei corpi di Leishman nel succo splenico pare che possano dedursi i seguenti fatti:

1° I corpi di Leishman presentando differente sviluppo dei loro elementi (nucleo, protoplasma, blefaroplasto) fanno pensare a successivi stadî di sviluppo.

2° La loro moltiplicazione avviene per divisione longitudinale rispettivamente del nucleo, del corpo cellulare e probabilmente del blefaroplasto.

3° La sede della moltiplicazione è il corpo delle grandi cellule della milza e di alcuni mononucleari.

4° La morte del parassita avviene per un processo di vacuolizzazione successivamente del corpo cellulare e del nucleo. Il blefaroplasto è l'ultimo a scomparire.

Ringrazio sentitamente il mio Maestro prof. Feletti per gli aiuti datimi

Catania, aprile 1910.

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FIG 1

FIG. 2

FIG. 3

FIG. 4

FIG. 5

FIG. 6

FIG. 7.

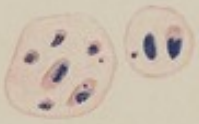

FIG. 8.

FIG. 9.

FIG. 10

FIG. 11.

FIG. 12.

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Reprinted for the Author from the BRITISH MEDICAL JOURNAL, March 16th, 1901.

THE APPLICATION OF ROMANOWSKY'S STAIN IN MALARIA.

BY MAJOR W. B. LEISHMAN, M.B., R.A.M.C.,

Assistant Professor of Pathology, Army Medical School.
[From the Pathological Laboratory, Netley.]

THE following note on the application of Romanowsky's method of staining for the demonstration of the presence of malarial parasites in the blood and, in particular, for the differential diagnosis of the tertian parasite, has been written in the hope that the simplified technique described below may render its great advantages more generally available to those who are brought into every-day contact with the disease.

The recent arrival at Netley of several large batches of invalids from India has given me the opportunity of using this stain in many cases of malaria, and the results obtained by it have proved strikingly superior to those in which other and better-known methods were followed, and I am convinced that those who may be led to give it a trial will appreciate the increased facilities it affords for the investigation of many important points in connection with the diagnosis, prognosis and treatment of malarial fevers. The method which has been followed in most of its essentials is that described with great care and detail by Dr. Georg Maurer[1] as the result of his work in Sumatra.

The chief advantages which the staining method described below holds over others are the following:

1. The specific action of the stain upon all red blood corpuscles infected by the tertian parasite.

2. The greater certainty of the detection of very young intracorpuscular forms of all varieties of the malarial parasite.

3. The facility with which the occurrence of a "mixed" infection may be detected, that is, the presence in the system simultaneously of more than one variety of the parasite.

4. After a little practice, the ease of its application and the certainty of the results obtained.

With regard to the first of these points—the specific action upon the red blood corpuscles infected by tertian parasites—the appearance in the infected cell, when stained by this method, of bright red dots or points was first pointed out by Schüffner.[2] A red cell infected by one of these tertian parasites forms one of the most striking of microscopical objects, as may be judged by a reference to Figs. 5-23 in the plate, which were all drawn from Nature from films prepared in the wards of this hospital and stained by Romanowsky's method.

The origin and nature of these dots are not by any means clear. The fact that their staining reaction is identical with that of the nuclear chromatin of the parasites might point to their being of similar structure; but, more probably, they are the result of trophic changes in the substance of the red cell brought about by the invading parasite, and are not directly connected with the parasite itself. Whatever their nature, they appear as fine more or less rounded granules scattered irregulary throughout the whole of the red cell, and, in the case of the more fully developed parasites, both over and underlying the parasite itself (see Figs. 5-23). They stain a deep ruby red colour, and are so numerous as to recall the fine eosinophile granules of the polynuclear leucocytes. Maurer lays stress on the fact that, from the time they make their first appearance, they increase in size only and not in number; but, in the forms sketched in Figs. 5-12—which show a very young stage of the tertian parasite, and were all taken from the same case—it will be noticed that the dots are both larger in size and fewer in number than those of another case (Figs. 13-23) in which the parasites had reached a more mature stage. In fresh unstained films and in those stained by other methods these dots were quite invisible.

The cases of malarial fever in soldiers which come under treatment in Netley are naturally of a very chronic type, and their differential diagnosis is rendered difficult by reason of previous prolonged treatment by quinine and other antiperiodics and, as I shall have occasion to refer to again, by the occurrence of mixed infections. It is, therefore, but rarely that a case can be classified accurately as quartan, tertian, or pernicious by means of the clinical signs alone, and in only one instance—in the case of a recrudescence of malarial fever in a patient invalided for another cause—was it found possible to infer the microscopical from the clinical signs. The appearance, however, of Schüffner's dots is so unique and, as far as my experience extends, so constant that I have little doubt that those who have the opportunity of applying this stain in cases of fresh infection in a malarious climate will find, as did Maurer under similar circumstances, that they are invariably present and occur only in red blood corpuscles infected by the tertian parasite.

Secondly, the advantages of a method which facilitates the detection of the youngest intracorpuscular forms of all varieties of malaria will also be obvious. With regard to the more mature intracorpuscular forms the examination of moist films or staining by any of the methods in general use demonstrates these with sufficient certainty, but with inferior detail of structure as compared with the method under consideration. It is, however, quite otherwise with the youngest forms. In illustration of this it may be mentioned that in two of the cases recently invalided from India, both markedly cachectic and liable to atypical attacks of pyrexia at irregular intervals, numerous observations of their blood by the ordinary methods yielded a constantly negative result, except that on one occasion a single crescent was detected. On the other hand, films made at the same moment, but stained in the manner described below, demonstrated the presence of numerous young undotted parasites in both cases; indeed, in one of them these were so abundant that a careful count was made, with the result that no less than 8 per cent. of the red cells were found to be infected, many having two distinct parasites (Fig. 4). Figs 1-4 and 24 were drawn from this case, and show very young ring-formed quartan parasites, and were selected to show the most usual

disposition of the small nucleoli of the parasite, which are deeply stained by this method. In the great majority of young forms there are two of these nucleoli, and they are invariably situated in the wall of the parasite and, most commonly, directly opposite to one another. Oval or pyriform rings are common, while the shape of the free amœbula, shown in Fig. 24, which is apparently attacking a normal red cell, is a frequent type.

Another point which this ready detection of young forms has enabled me to note is that in every case in which the parasite was found to be present during or immediately preceding an attack, an examination of the patient's blood at any period of the attack or between the attacks has given a similar positive result, though quantitatively less, while by other methods, applied simultaneously, negative results were recorded on many occasions.

It was also noted in several cases that these young forms, though at times very numerous, did not reach maturity, and in such cases one or two red-stained dots were often seen lying on or in red cells apparently unaffected by parasites. These dots recalled the nucleoli figured in 1-4, and may possibly have been the remains of a partially-destroyed parasite. These cases were at the time being treated with quinine.

There are many other points of great interest in connection with the persistence of these young immature forms in the blood and their bearing upon prognosis and upon the effects of quinine and other remedies, but the number of my observations is at present insufficient to generalise from; and it is in the hope that others, more favourably situated with regard to clinical material, may be led to try this method of staining that I have been led to give a description of its technique.

Thirdly, in the detection of the "mixed" forms of malarial infection great help is obviously to be had from any method by which one variety is marked out sharply and definitely from the others. The presence of Schüffner's dots in the red cells infected by the tertian parasite (Figs. 5-23) does for this variety of malaria what the presence of crescent forms does for the variously named remittent, pernicious, or æstivo-autumnal, and, if we accept the classification which appears to find most favour at the present moment, there remains but the quartan in which the proved absence of crescent forms or of infected red cells containing Schüffner's dots suffices to distinguish it from the others. The criteria at our command for the differential diagnosis of the varieties of malarial fever, other than this selective staining reaction—namely, the clinical characteristics of the fever, the size of the infected red blood corpuscles, the darker or lighter shade of the melanin granules, the character of the amœboid movements of the parasite, and the number of spores contained in the mature sporocyte—form a collection of points which it is far from easy to determine for every case, and which involve the preparation and examination of both fresh and dry films at several stages of the attack. On the other hand, as far as my observations go, Romanowsky's stain rarely fails to demonstrate the nature of the parasite or the existence of a mixed infection on a single examination of a properly prepared and stained film. Of the cases of malaria at Netley on which I have tested this method the larger number proved to be mixed infections—as, indeed, one would expect from their atypical clinical features and their chronic and intractable nature—and, of these, the most frequent combination was that of the tertian and the pernicious forms.

In proceeding now to a description of the preparation and application of Romanowsky's stain for the purpose of demonstrating the presence and nature of malarial infections of the blood the method described by Maurer has been fairly closely followed, and, in the points in which the technique described below differs from his, it is not to be understood that I consider these departures better, but that they, I trust, render the process somewhat easier of application and more capable of being carried to a successful issue by those whose microscopical outfit is limited.

Preparation of the Blood Film.

A series of cover glasses, well polished and perfectly free from grease, are laid on a clean sheet of paper close to the patient, and blood films prepared as follows: Care should be taken that the cover glasses are never subsequently handled except with a pair of forceps, as the slightest trace of moisture from the fingers will endanger the result. Any method which ensures a thin even film may be used, but perhaps the easiest is the following: A few strips of very thin tissue paper—cigarette paper answers admirably—are cut into rectangular slips 2 inches by ½ inch. A drop of blood the size of a pin's head is expressed from a needle prick in the patient's finger and lightly touched by the end of one of the paper slips. The edge of the paper bearing the droplet of blood is then quickly applied to a cover glass and when the drop has spread itself out between the glass and the paper the latter is gently and lightly drawn across its surface. This will be found to leave a very thin and evenly-distributed film. Unless pains are taken by this or some similar method to secure a thin even film, in which the red blood corpuscles are well spaced out and not overlying one another, good results are not to be expected. A series of films having been prepared in this way are allowed to dry in the air, or the drying may with benefit be hastened by gentle warming.

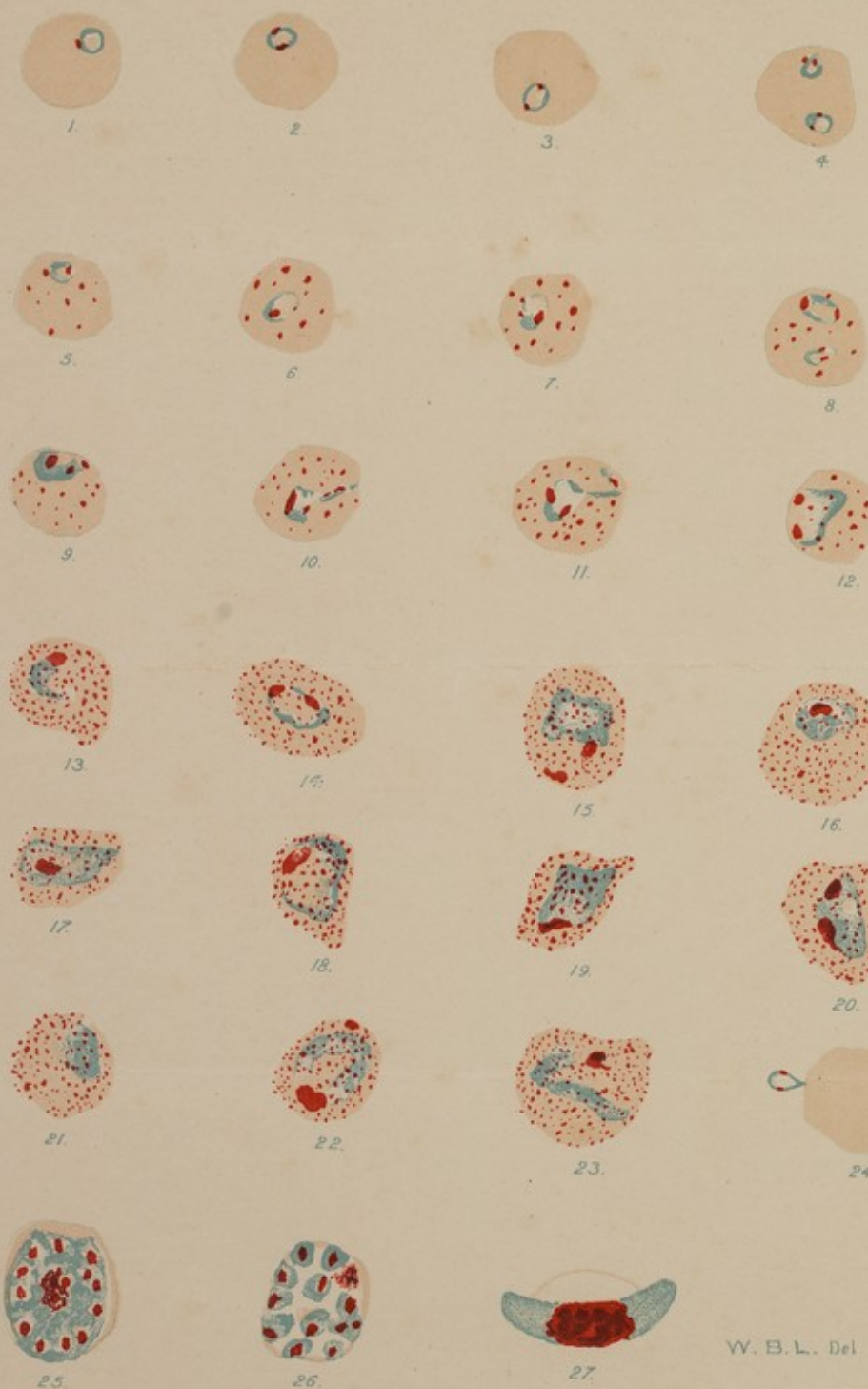
Fixing.—Immerse the films for from two to five minutes in a mixture of equal parts of ether and absolute alcohol, or, as as an alternative if ether be not available, as may happen in a hot climate, in absolute alcohol alone for ten minutes. Fixing solutions containing perchloride of mercury are unsuitable, while fixation by heat is troublesome and presents no advantages over the first method. After removing the film from the fixing solution, wash it well in water and allow it to dry in the air before staining. The shorter the intervals between the preparation and fixation of the films and between the fixation and the staining the better are said to be the results, but I have found that unfixed films two months old may be successfully stained by slightly increasing the period of immersion in the dye. In any case, films must not be fixed until immediately before they are stained.

Staining.—The effects produced by Romanowsky's staining are due to a red dye which is extracted from alkaline solutions of certain kinds of methylene blue when to such a solution is added a small quantity of a very weak watery solution of eosin, and the evidences of the advent of this red dye are found in the appearance on the surface of the mixture of a a brilliant metallic scum, a certain amount of precipitation and the staining of the walls of the containing glass vessel, or of any glass object dipped in the mixture, of a definitely red tint. This red dye is called by German authors "Rot aus Methylenblau," and in its extraordinary affinity for nuclear chromatin lies its great value as a differential stain for the malaria parasite, and also for demonstrating the structure of bacteria. That it is a new product, evolved by the interaction of the eosin and methylene blue and is not due to the weak eosin itself, is easily seen by the failure of its appearance when an ordinary watery solution of methylene blue is substituted for the special alkaline solution of which the formula is given below. Only a few varieties of methylene blue will produce this red dye in sufficient quantities, and Maurer gives a list of those with which he has obtained the best results, as well as details of his method of titrating one against the other so as to obtain the maximum staining power; but for the purpose of this article it will suffice to describe only the solutions with which I have worked, and which have yielded equally satisfactory results in other hands in this laboratory. Both the dyes described below were obtained from the firm of Dr. G. Grübler and Co. (Leipzig). Should these particular dyes be unprocurable, the proper proportion of eosin to be added to the solution of methylene blue must be determined by experiment.

Solution A.—"Medicinal" methylene blue. A 1 per cent. solution of this is made in distilled water, and then rendered alkaline by the addition of 0.5 per cent. of sodium carbonate. It is necessary before use that this solution should be heated for some time, and this may be conveniently done by leaving it in a tropical sun for two days or in a warm room for a week. To prevent the growth of moulds 0.25 per cent. of formalin may be added. It is recommended that after heating it should be allowed to stand for a week or two before use.

Solution B.—"Eosin extra B.A." A 1-1,000 solution in distilled water.

These concentrated stock solutions A and B, preserved in stoppered glass bottles, will keep indefinitely. When fixed blood films are to be stained, a portion of each of these solutions A and B is further diluted with distilled water in the proportion of 1 to 25, and kept ready in a couple of graduated glasses. An equal volume of each diluted solution is now poured on to the cover glass in such a way that they come in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W. B. L. Del.

contact with the film at the moment of mixing. The reason for so doing is that it is at this moment that the production of the red dye is greatest and that it acts with most intensity. A convenient way of carrying this out is to pour a measured quantity of each—say 2 c.cm.—into two watch glasses, the cover glass being placed, film uppermost, in a third empty watch glass. The glasses containing the diluted A and B solutions are then picked up in either hand and their contents poured simultaneously upon the film. To ensure intimate mixture of the two solutions, the cover glass is now picked up with a pair of forceps and used to stir the mixture; it is then replaced, film downwards, at the bottom of the watch glass, and allowed to stain at room temperature for from half to one hour. Longer staining than this or heating are undesirable, and add nothing to the clearness of the result.

At the end of half an hour the film is removed from the stain and examined in water under a power of ⅛ or ¼ inch, with a view to seeing whether the red dye has acted sufficiently intensely. The index to this is found first in the leucocytes, whose nuclei should appear sharply defined and stained an intense ruby-red colour; secondly, in the blood plates, which will at once attract attention, being stained sharply and of the same deep tint as the nuclei of the leucocytes. If instead of this deep-red colour the nuclei and blood plates are only purple or deep violet, the staining is insufficient, and the films must be replaced in the solution for a short time. A certain amount of deposit will also have taken place, but this is easily got rid of subsequently in the process of decolorisation.

The point to which I have indicated the staining should be carried represents a slight degree of overstaining, as it is simpler to overstain and then decolorise than to stop the action of the dye at the right moment. Several methods of carrying out this decolorisation have been advocated, of which two are at the same time simple and efficacious. First, rapid method; the film after washing in water is rinsed in absolute alcohol for two or three seconds, then immediately transferred to water and all traces of alcohol removed. Second, slow method; in this water alone is used and the decolorisation may be carried out either by holding the film under a tap of running water for some time, or by leaving it to soak in water for from half an hour to one hour. In either case the extent of the decolorisation is controlled by examination under the microscope from time to time. Whichever method be adopted the appearance of the film when it is ready to mount should be as follows: The red cells are transparent and nearly colourless, or perhaps slightly tinged green or pink. The polynuclear leucocytes have their nuclei stained ruby red or magenta, the extranuclear portion being colourless or containing a few reddish granules. The nuclei of the mononuclear leucocytes and lymphocytes are of the same colour as those of the polynuclears, and their margins are very clearly defined, while the extranuclear portion is stained a beautiful eau-de-nil or pale blue colour. The blood plates are ruby red, with definite involuted margins.

Mounting.—The films are now dried in the air or by gentle heat, and mounted for examination. Unfortunately, Canada balsam has not proved a very good mounting medium, as it detracts from and alters to a considerable extent the brightness of the colouring described above, and I have not, so far, been successful in finding a suitable substitute. However, either it or cedar oil may be used for the purpose without interfering with the results other than those of an æsthetic nature.

In conclusion, I wish to express my thanks to Civil-Surgeon G. H. B. Harvie, M.B., for the assistance he has afforded me in the clinical examination of many of these cases.

References.

[1] Die Tüpfelung der Wirtzelle des Tertianaparasiten, Dr. Georg Maurer, *Centralbl. für Bakt.*, vol. xxviii, Nos. 4 and 5. [2] Beitrag zur Kenntniss der Malaria, Dr. W. Schüffner, *Deutsch. Archiv f. klin. Med.*, vol. lxiv.

Description of Coloured Plate.

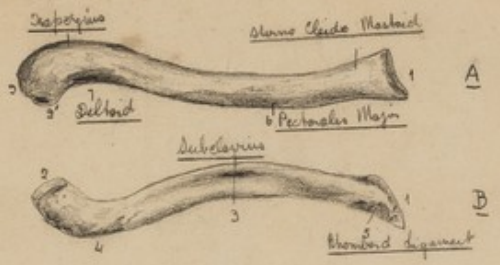
Figs. 1-4.—Young intracorpuscular forms of the quartan parasite.
Fig. 1.—Circular ring-form with single nucleolus.
Fig. 2.—Oval form with two nucleoli situated laterally.
Fig. 3.—Oval form with two nucleoli situated at the poles.
Fig. 4.—Red cell infected with two parasites.
Figs. 5-7.—Young forms of the tertian parasite showing gradual enlargement and alteration of the shape and position of the nucleolus; also the presence of Schüffner's dots throughout the red cell, even in the youngest form—No. 5.
Fig. 8.—Red cell infected by two young tertian parasites.
Figs. 9-12.—More fully-developed tertian parasites, in 10 and 11 showing amœboid processes.
Figs. 13-23.—Various forms of tertian parasites approaching maturity, taken from another case, and showing more numerous and finer dots; also enlargement and altered contour of the infected red cells. In Figs. 15, 22, and 23 the enlarged nucleoli are apparently dissociated from the parasite, and free in the substance of the red cell.
Fig. 24.—A free amœbula attacking a healthy red cell.
Figs. 25 and 26.—Two stages in the formation of the mature quartan sporocyst or rosette.
Fig. 27.—Crescent form of pernicious malaria showing the melanin granules almost masked by a mass of nuclear chromatin.

Folkestone
[Ostend?]
[via?] Frankfort
[Basle?]
Lucerne
Interlaken
Chamonix [illegible]

Return by London
Southampton

William. B. Leishman.

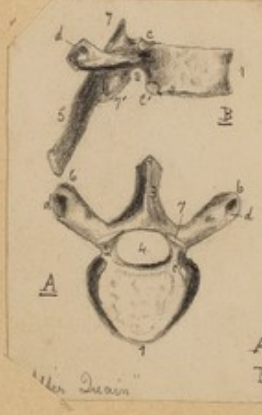
THE CLAVICLE or COLLAR BONE



A. The Right Clavicle from above.-
B. The Right Clavicle from below.-

Explanatory.
1. Sternal end.-
2. Acromial end.- 2' Facet for artic" with acromion
3. Groove for subclavius muscle.-
4. Elevation for Conoid. Clavicular ligaments.-
5. Depression for Costo-Clavicular & Rhomboid ligam"
6. Pectoralis major muscle.-
7. Deltoid muscle

The Sixth Dorsal Vertebra

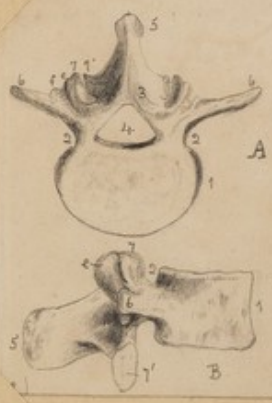


1. The body.-
2. The Pedicle.-
3. The Lamina.-
4. The Ring.-
5. Spinous Process.-
6. Transverse Process.-
7.7' Superior & Inferior Articulating Processes.-
c.c' Sup' & Inf' facets for artic" of head of rib.-
d. Facet for artic" of tubercle of rib.-

A Viewed from above.
B Viewed from right side.

"Allen Drain"

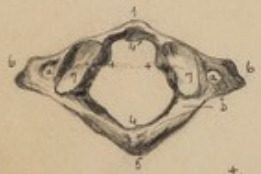
The third Lumbar Vertebra



1. The body.-
2. Pedicle & Intervertebral Notch.-
3. The Lamina.-
4. The ring (triangular).-
5. The Spinous Process.-
6. Transverse Process.-
7.7' Supra' & Infra' Articulating Processes.-
e. Mamillary Tubercle.-
f. Accessory Tubercle.-

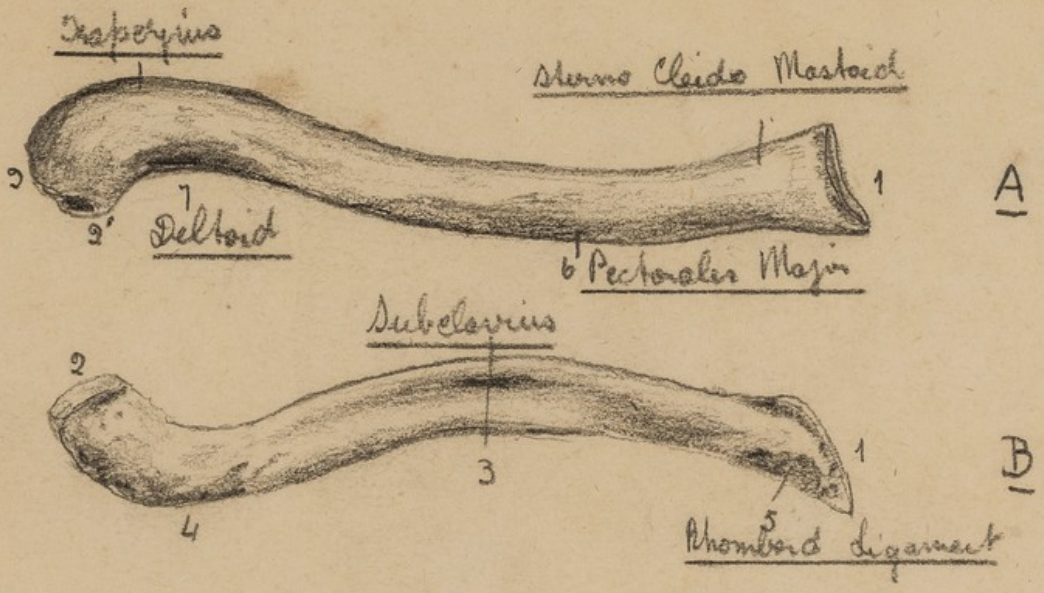
A Viewed from above.
B. Viewed from right side

The Atlas Vertebra from above.



1. The Anterior arch with a tubercle in front.
4. The posterior part of the ring.-
5. Indication of Spinous tubercle.-
4'. The anterior part of ring containing the odontoid process.-
6. Transverse processes.
7. The condyloid artic" processes.-
+ Tubercles to which transverse lig" is attached.
a. Foramen in transverse processes
b. Groove for Vertebral Artery

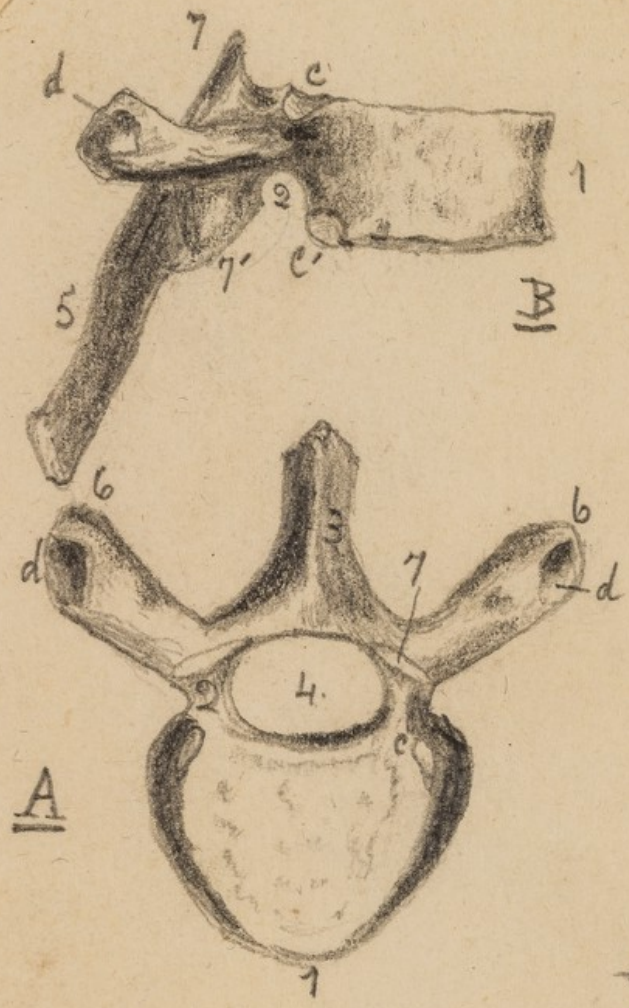
THE CLAVICLE or COLLAR BONE



A. The Right Clavicle from above.-
B. The Right Clavicle from below.-

Explanatory.

1. Sternal end.-
2. Acromial end.- 2' Facet for artic with acromion
3. Groove for subclavius muscle.-
4. Elevation for Coraco-Clavicula ligaments.-
5. Depression for Costo-Clavicula & Rhomboid ligam
6. Pectoralis major muscle.-
7. Deltoid muscle.



The Sixth Dorsal Vertebra

1. The body.–
2. The Pedicle.–
3. The Lamina.–
4. The Ring.–
5. Spinous Process.–
6. Transverse Process.–
7, 7' Superior & Inferior Articulating Processes.–

c. c' Sup.r & Inf.r facets for artic.n of head of rib.–

d. Facet for artic.n of tubercle of rib.–

A Viewed from above.

B Viewed from right side.

"Ulster Dream"

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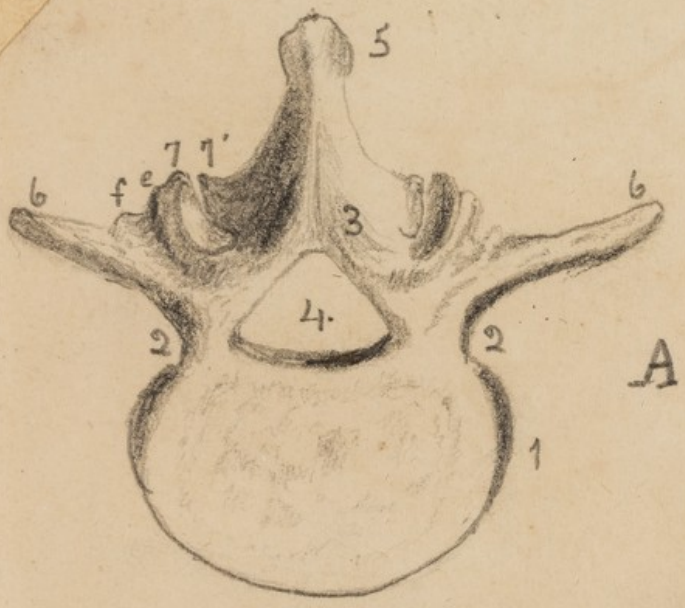
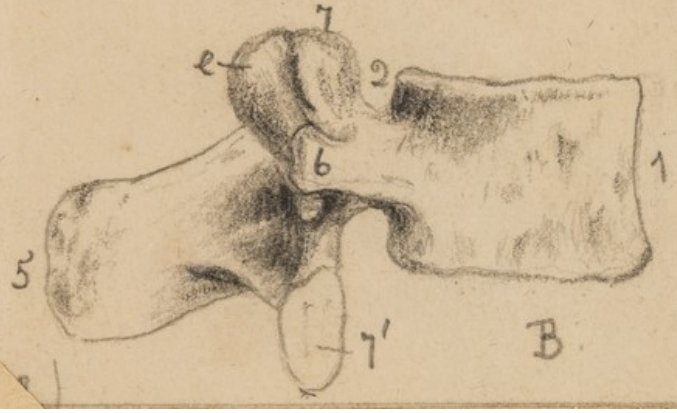
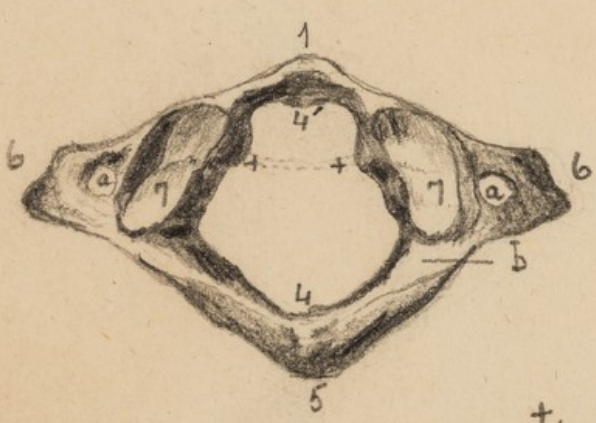




The third Lumbar Vertebra

1. The body.—
2. Pedicle & Intervertebral Notch.—
3. The lamina.—
4. The ring (triangular).—
5. The Spinous Process.—
6. Transverse Process.—
7.7' Super^r & Infer^r Articulating Processes.—
e. Mamillary Tubercle.—
f. Accessory Tubercle.—

A Viewed from above.
B. Viewed from right side



The Atlas Vertebra from above.

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4. The posterior part of the ring.-
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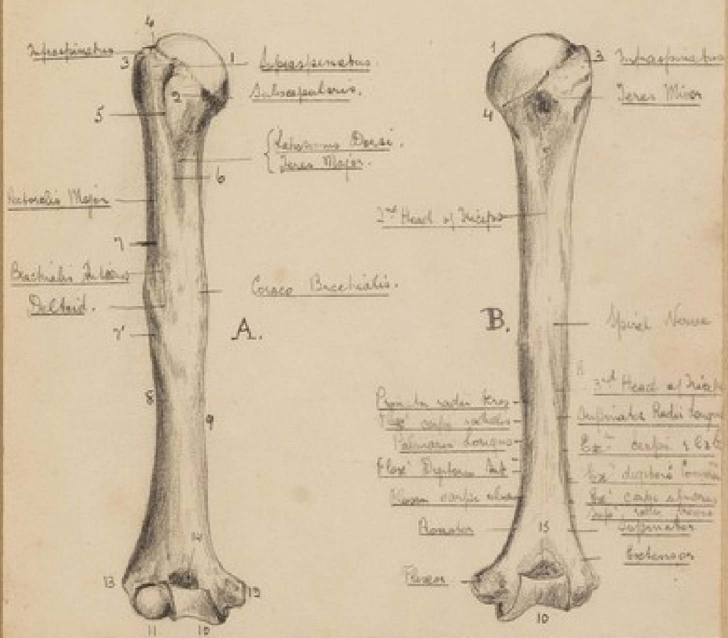
4'. The anterior part of ring containing the odontoid process.-
6. Transverse processes.
7. The condyloid articular processes.-
+. Tubercles to which transverse lig.ts is attached.
a. Foramen in transverse processes
b. Groove for Vertebral artery.

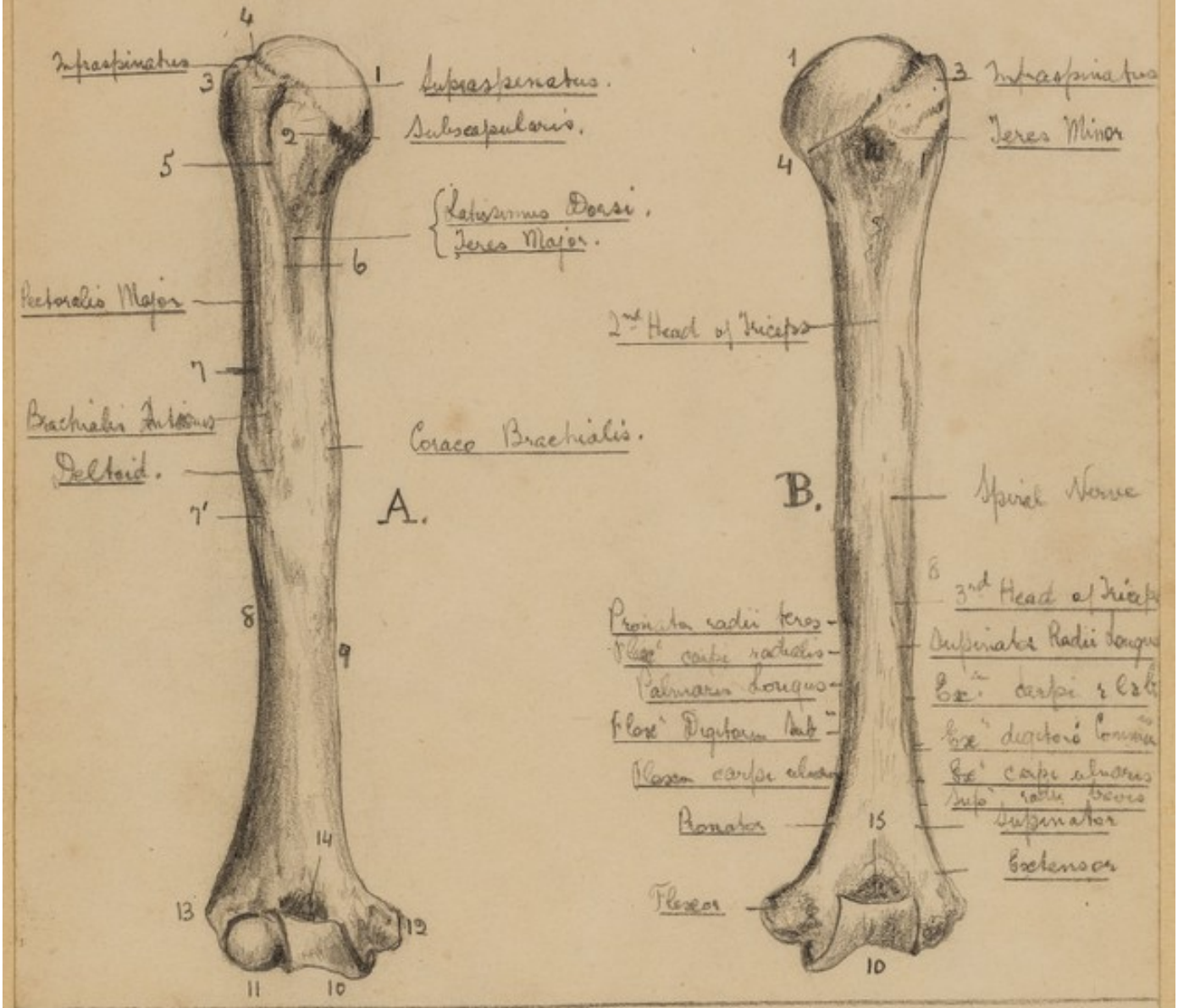
THE HUMERUS or ARM BONE



A. Right Humerus from before.
B. Right Humerus from behind.

1. — The Articular Head.—
2. — The Lesser Tuberosity —
3. — The Greater Tuberosity —
4. — The Neck.—
5. — The Bicipital Groove —
6. — Inner Bicipital Ridge g.a.t. latissimus dorsi & teres major
7. — Outer Bicipital Ridge g.a.t. pectoralis major —
7'. — Triangular mark of insertion of deltoid.—
8. — Spiral Groove — 9 — Inner ridge of humerus
10. — Trochlear Articular surface.— 11 — Capitellum radial condyle —
12. — Epitrochlea & internal supracondyloid eminence
13. — Capitellum & external s. c.
14. — Coronoid Fossa — 16. Olecranon Fossa

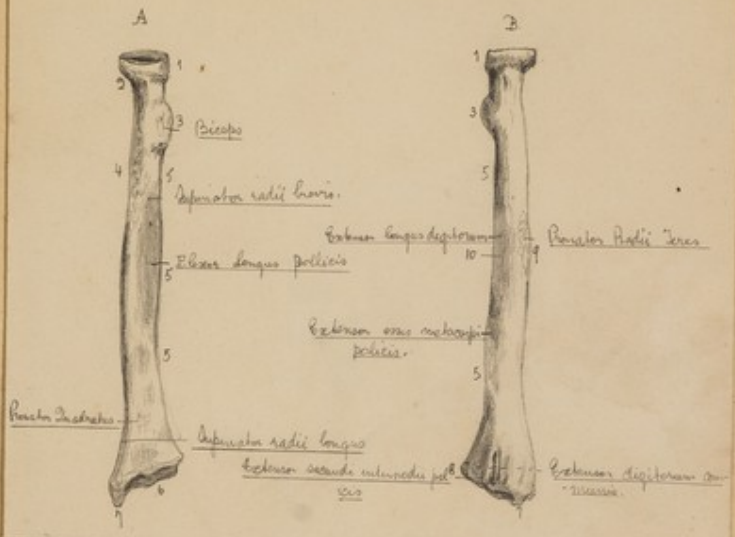
THE HUMERUS or ARM BONE



A. Right Humerus from before.
B. Right Humerus from behind.

1. The Articular Head.-
2. The Lesser Tuberosity.-
3. The Greater Tuberosity.-
4. The Neck.-
5. The Bicipital Groove.-
6. Inner Bicipital Ridge g.a.t. latissimus dorsi, teres major
7. Outer Bicipital Ridge. g.a.t. pectoralis major.-
7'. Triangular mark of insertion of deltoid.-
8. Spiral Groove.- 9. Inner ridge of humerus
10. Trochlear Articular surface.- 11. Capitellum. radial condyle.-
12. Epitrochlear = internal supracondyloid eminence
13. Capitular = external S.-e.-
14. Coronoid Fossa.- 15. Olecranon Fossa

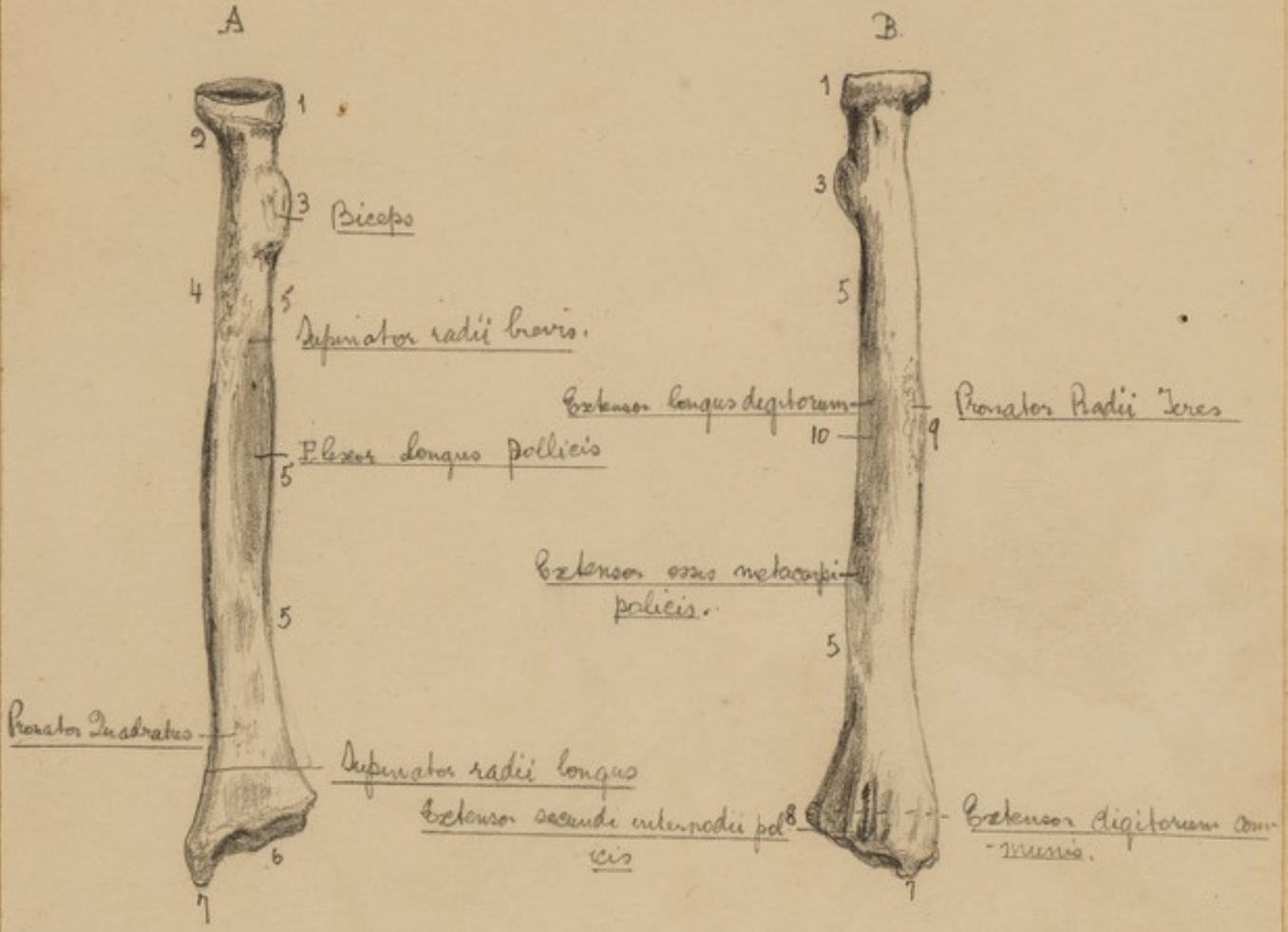
THE RADIUS



A. Right Radius from before.
B. Right Radius from behind.

1. Head, with hollow for humerus, and surface for articulation with ulna
2. The neck
3. The tubercle of the radius.
4. Opposite the oblique line
5. Interosseous ridge.
6. Carpal articular surface.
7. Styloid process.
8. Articular hollow for low end of ulna.
9. Pronator radii teres muscle.
10 + 11. Muscles

THE RADIUS



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B. Right Radius from behind.

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2. The neck.
3. The tubercle of the radius.
4. Opposite the oblique line.
5. Interosseous ridge.
6. Carpal articular surface.
7. Styloid process.
8. Articular hollow for low end of ulna.
9. Pronator radii teres muscle.
10 + 11. Muscles

THE ULNA.

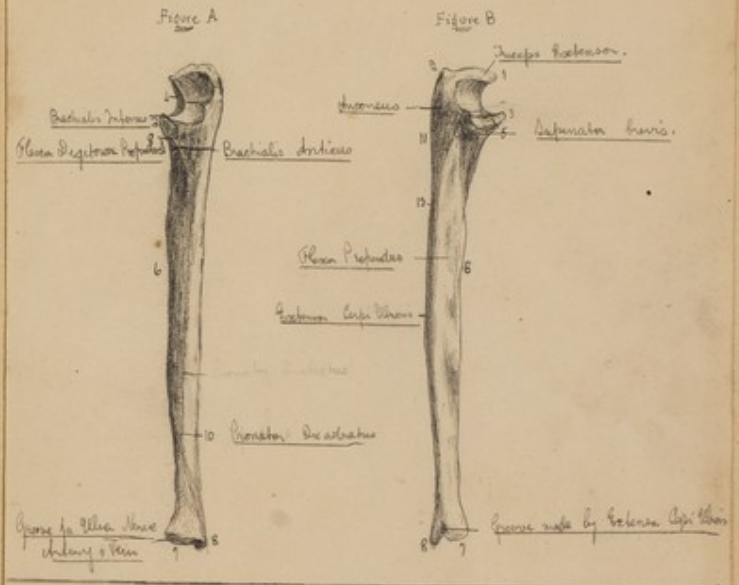


Fig A. Right Ulna from before
Fig B. Right Ulna from behind } reduced.

1 — Point of olecranon.-
2 — Tuberosity of the olecranon.-
3 — End of coronoid process.-
4 — Cresta sigmoid articular surface.-
5 — Fossa sigmoid cavity.-
6 — External Border & interosseous ridge.-
7 — Fossa ostensity on head.-
8 — Styloid process.-
9 — Brachialis Anticus muscle, below coronoid process.-
11 — Anconeus muscle.-
12 — Smoothed by extensor muscles of fingers.-

THE ULNA

Figure A

Figure B

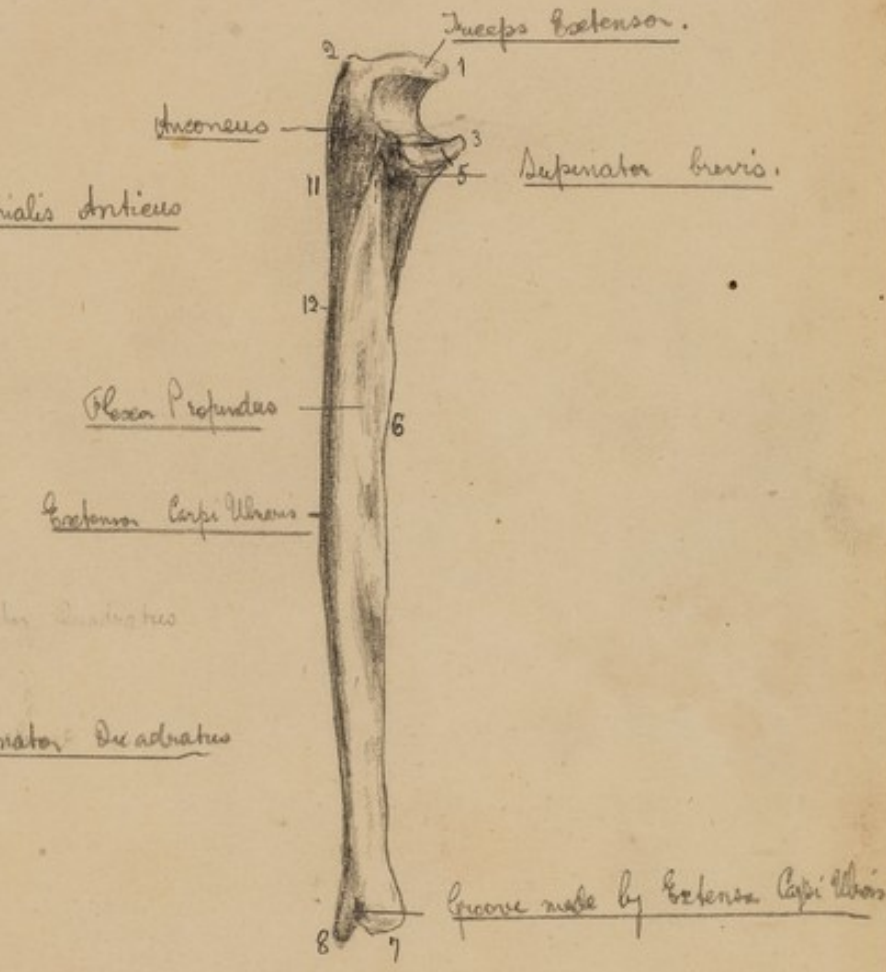
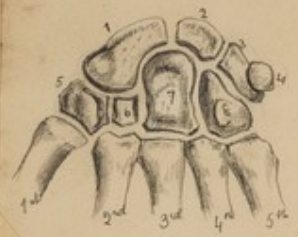


Fig A — Right Ulna from before
Fig B. — Right Ulna from behind & without.

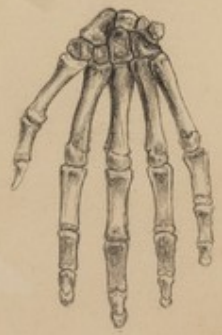
1. — Point of olecranon.-
2. — Tuberosity of the olecranon.-
3. — End of coronoid process.-
4. — Greater sigmoid articular surface.-
5. — Lesser sigmoid cavity.-
6. — External border & interosseous ridge.-
7. — Lower extremity or head.-
8. — Styloid process-
9. — Brachialis anticus muscle, below coronoid process.-
11. — Anconeus muscle.-
12. — Smoothed by extensor muscles of fingers.-

THE CARPUS

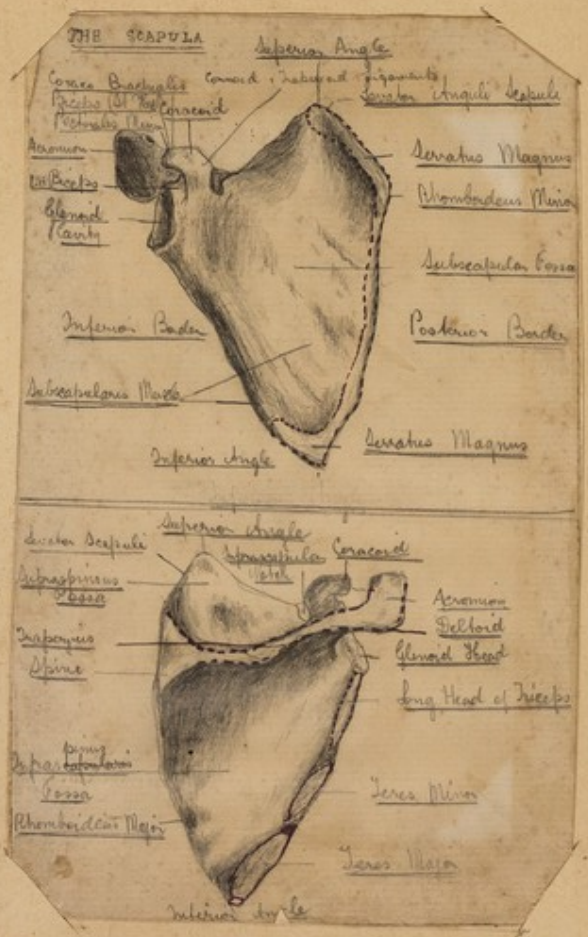


1. The Scaphoid (Scaphoideum)
2. Semilunar (Semilunare)
3. Cuneiform (Cuneiforme)
4. Pisiform (Pisiforme)
5. Trapezium (Multangulum majus)
6. Trapezoid (Multangulum minus)
7. Os Magnum (Magnum)
8. Unciform (Hamatum)

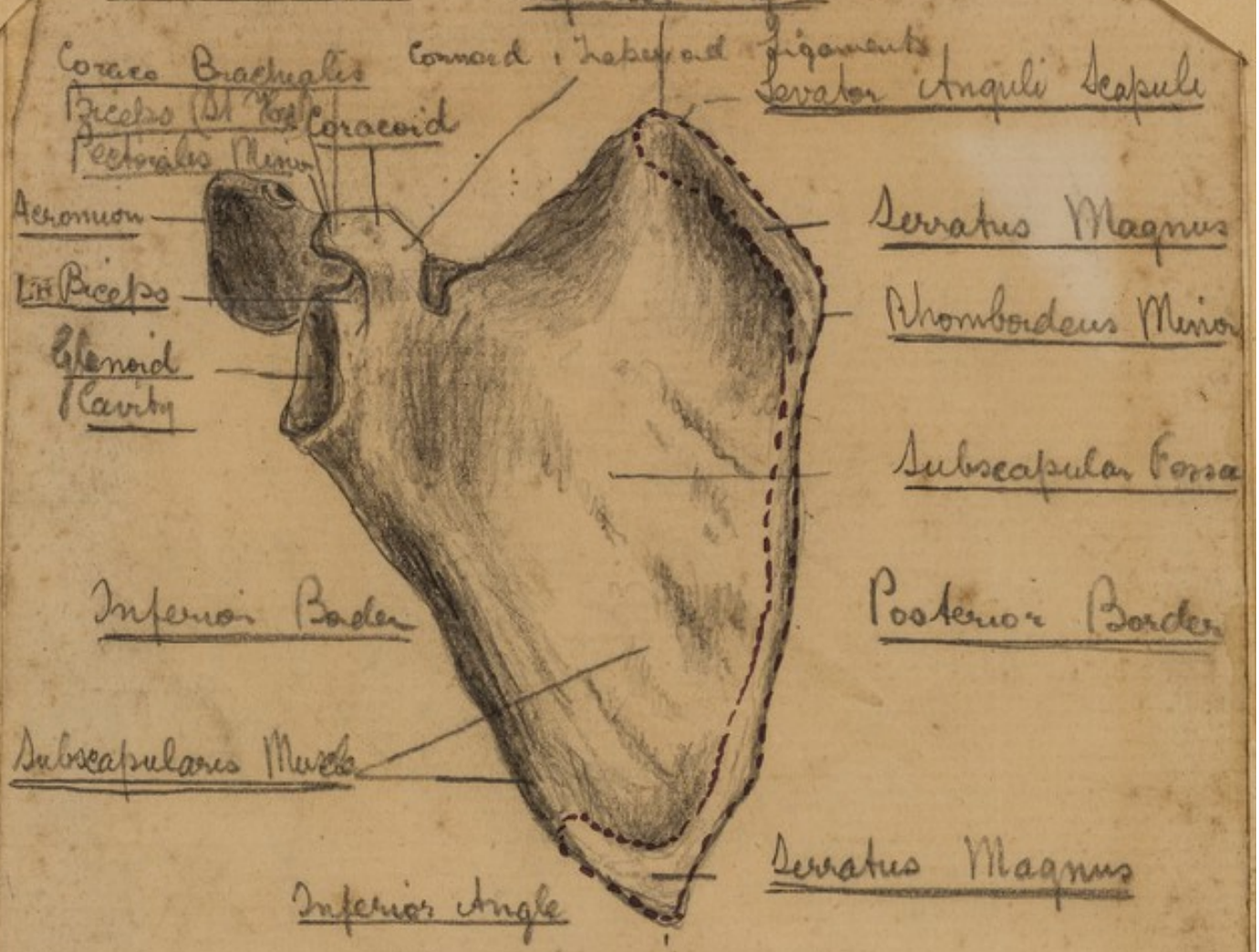
THE HAND.

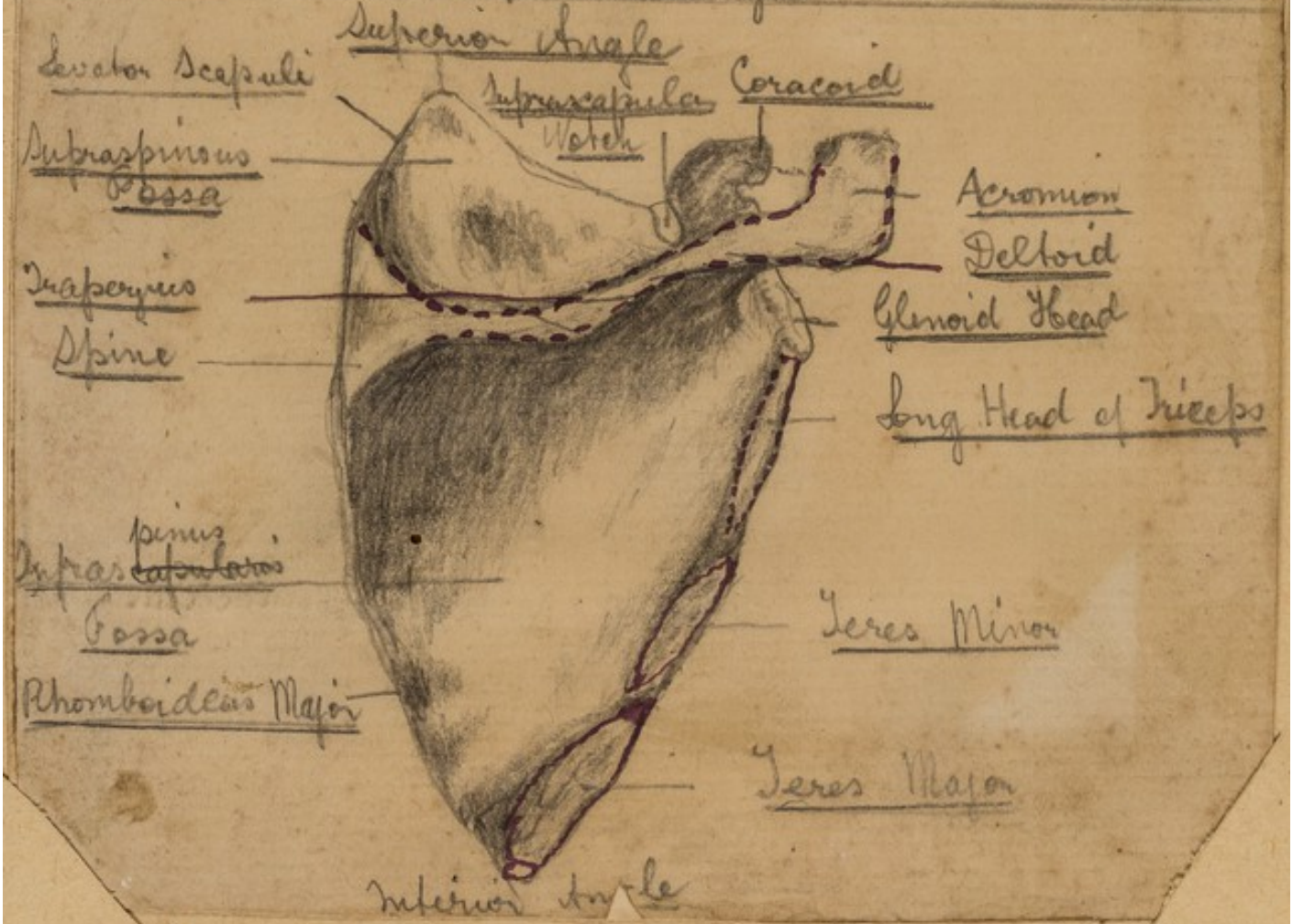


THE SCAPULA

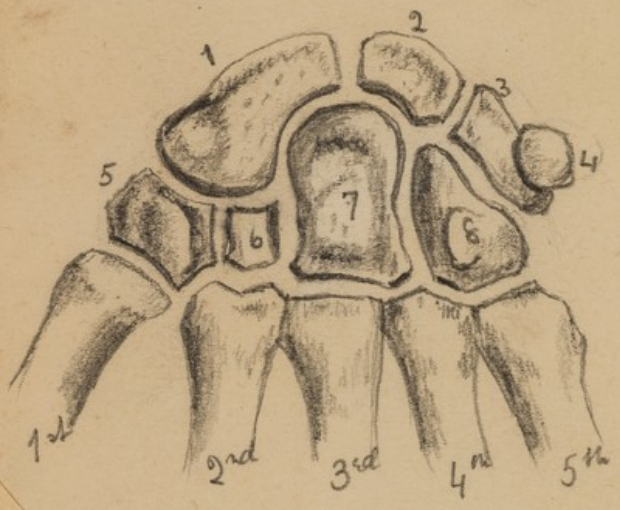


THE SCAPULA


Superior Angle
Coraco Brachialis
Biceps (Sh Head)
Pectoralis Minor
Coracoid
Conoid + Trapezoid ligaments
Levator Anguli Scapulæ
Acromion
Serratus Magnus
Lg Biceps
Rhomboideus Minor
Glenoid Cavity
Subscapular Fossa
Inferior Border
Posterior Border
Subscapularis Muscle
Serratus Magnus
Inferior Angle

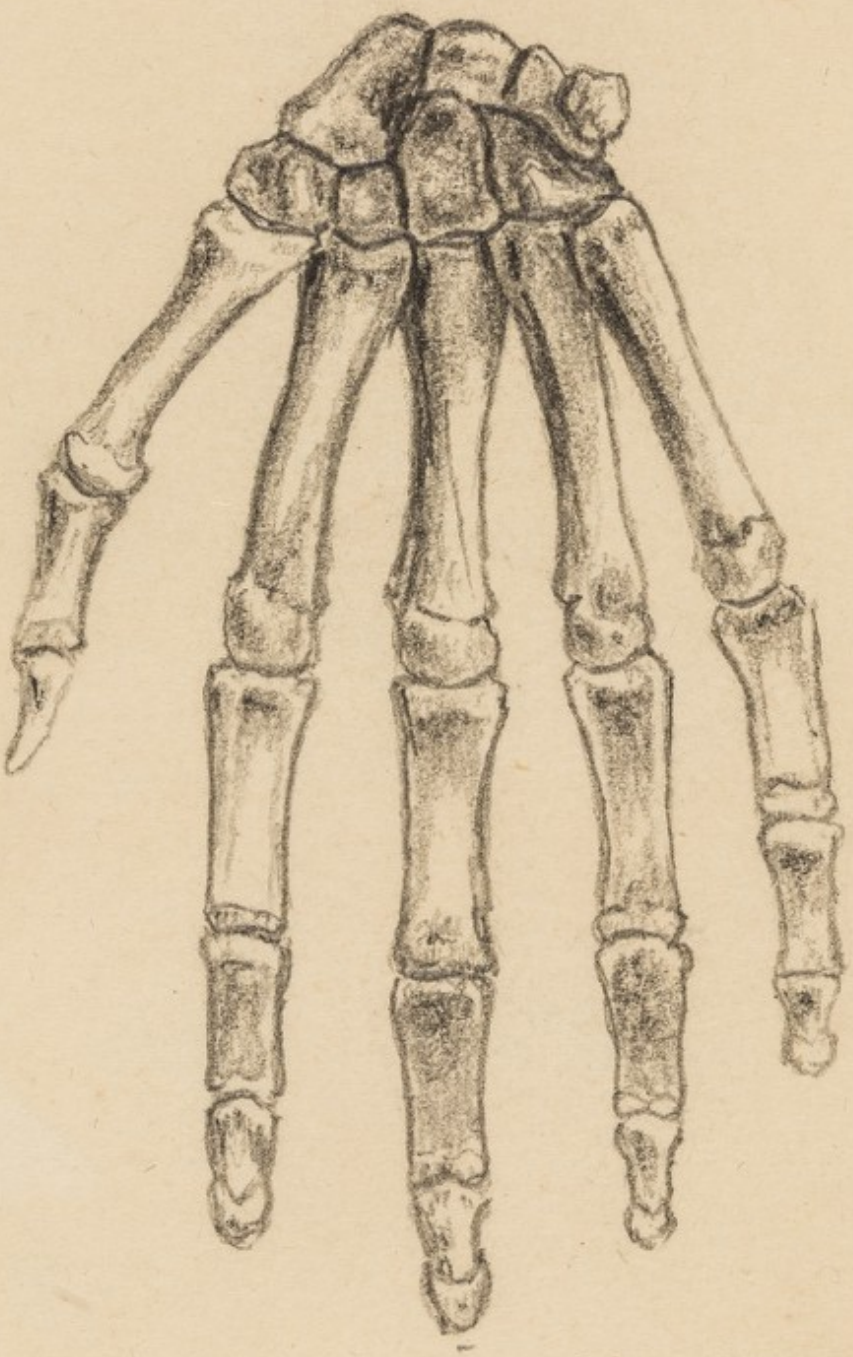

Superior Angle
Levator Scapulæ
Supraspinous Fossa
Coracoid
Suprascapular Notch
Acromion
Deltoid
Trapezius
Spine
Glenoid Head
Long Head of Triceps
Infraspinous Fossa
Teres Minor
Rhomboideus Major
Teres Major
Inferior Angle

THE CARPUS



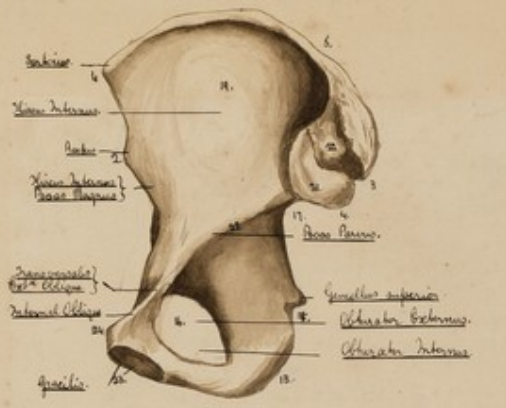
1. The Scaphoid (5 articulations)
2. Semilunar (5 artns)
3. Cuneiform (3 artns)
4. Pisiform (1 artn)
5. Trapezium (4 artns)
6. Trapezoid (4 artns)
7. Os Magnum (7 artns)
8. Unciform (5 artns)

THE HAND.

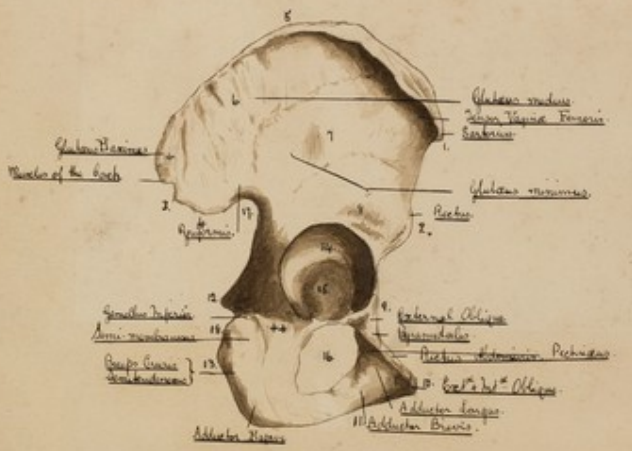


Right Os Innominatum from the dorsal aspect.
+ from the Inner or Pelvic surface.

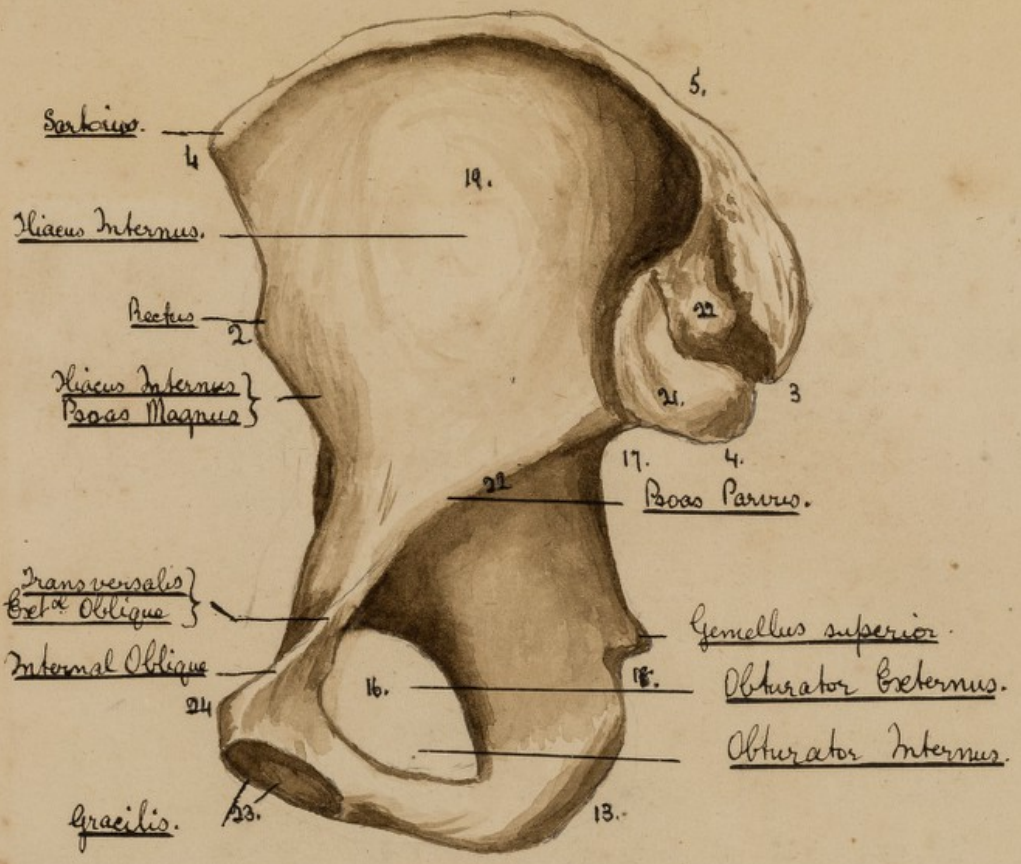
1. Anterior superior spinous process.-
2. Anterior inferior do -
3. Posterior superior do -
4. Posterior inferior do -
5. Crest of the ilium.-
6. Surface occupied by gluteus medius.-
7. Above 3. gut. gluteus maximus.-
8. Between the 2 curved lines gut. gluteus minimus.
9. Groove occupied by the posterior tendon of the rectus femoris.-
10. Superior ramus of the pubis and pectineal line.
11. Crest + spinous process of the pubis.-
12. Junction of descending ramus of pubis with the ascending ramus of the ischium.-
13. Spine of the ischium.-
14. Tuberosity of the ischium.-
15. Cartilaginous surface of the acetabulum.-
16. Synovial depression, and pit for ligament.-
17. Thyroid or obturator foramen.-
18. Greater sciatic notch.-
19. Lesser sciatic notch.-
20. Groove for obturator externus.-
21. Iliac fossa.-
22. Ilio pectineal line, or brim of true pelvis.-
23. Auricular cartilaginous sacro-iliac surface.-
24. Rough surface for "post." sacro-iliac ligaments.-
25. Oval surface of the symphysis pubis.-
26. Spinous process of the os pubis, ending the ilio-pectineal line + the crest of the pubis.-



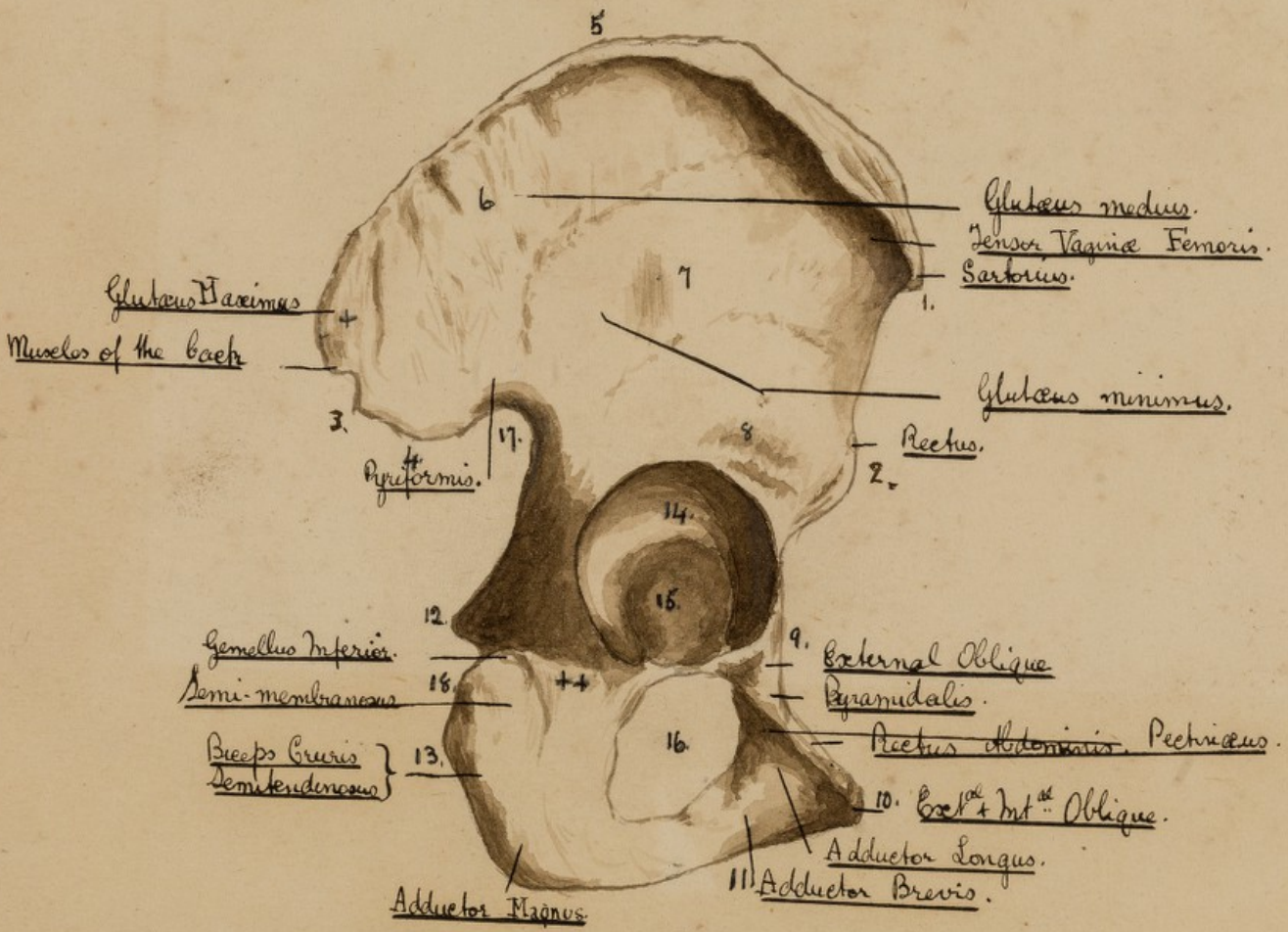
Right Os Innominatum from the Pelvic or Inner surface



Right Os Innominatum from the dorsal aspect.



Right Os Innominatum from the Pelvic or Inner surface.

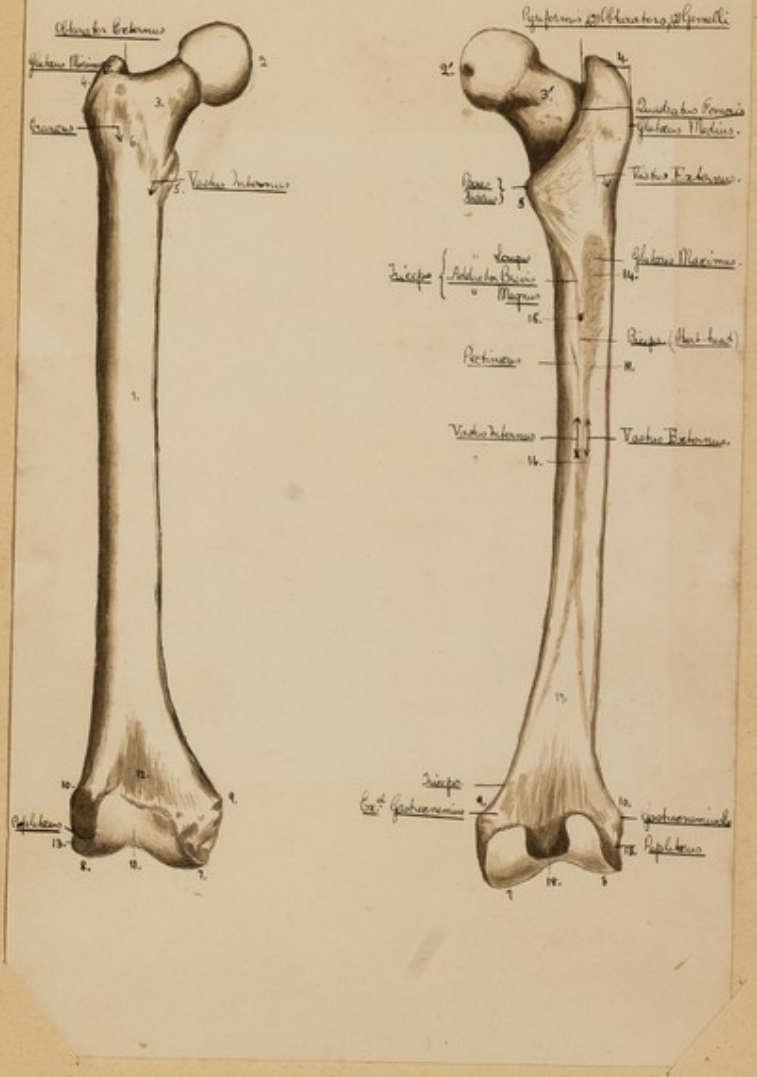


Right Os Innominatum from the dorsal aspect.

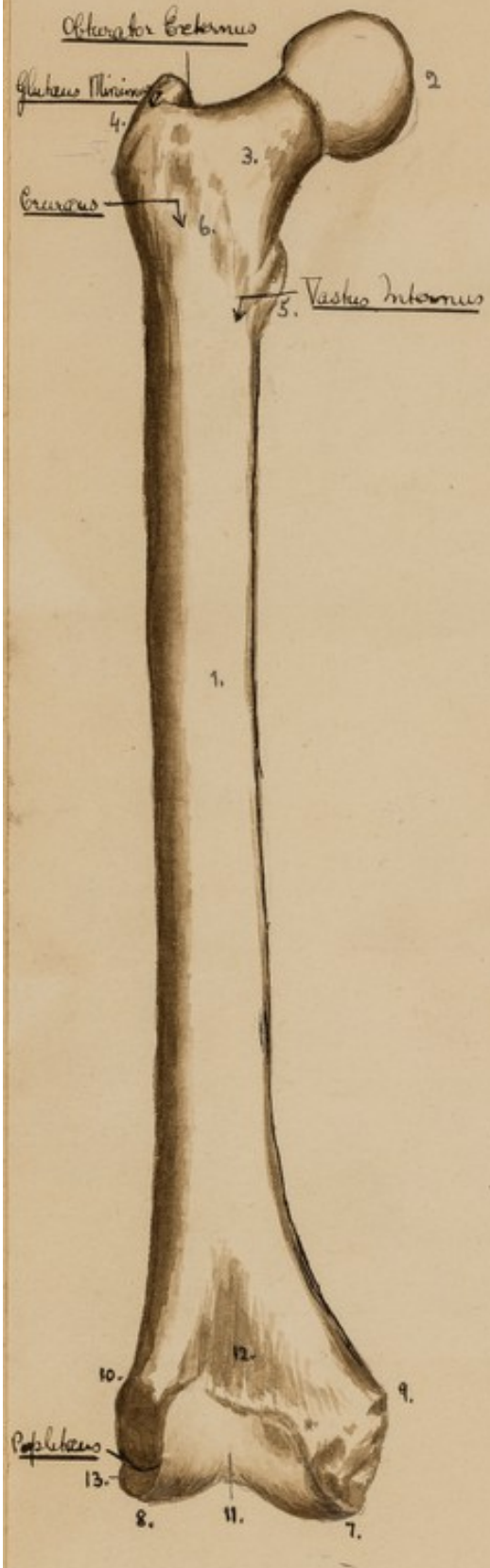
Femur of a male from before & from behind

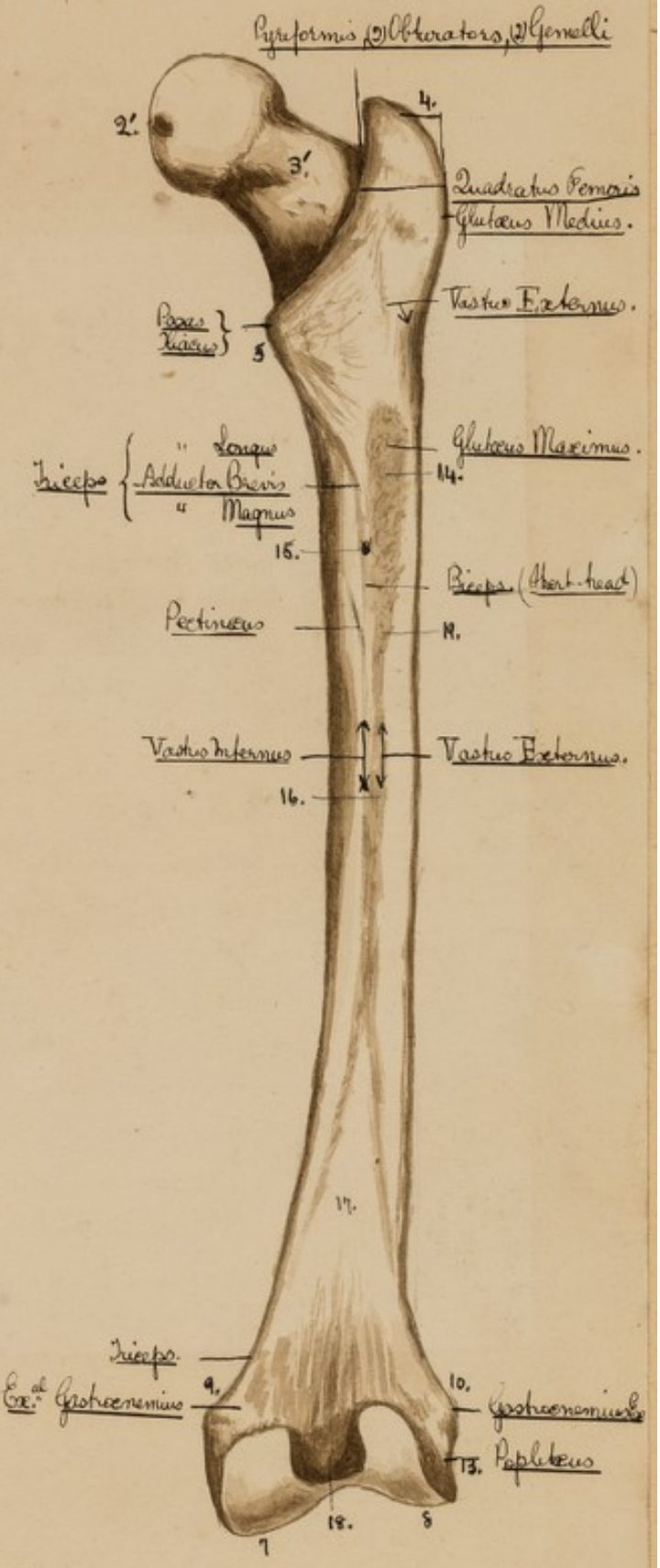
1. Shaft.-
2. Head.- 2'. Pit for ligamentum teres.
3. Neck.- 3'. Digit groove of obturator externus.
4. Great trochanter.-
5. Less trochanter.-
6. Anterior inter trochantoric line.-
7. Internal articular condyle.-
8. External ditto.-
9. Internal tuberosity.-
10. External do.-
11. Patellar articular surface.-
12. Infrapatellar surface.-
13. Groove for the tendon of popliteus.-
14. Rough for glutaeus maximus.-
15. Two lines running from linea aspera to the small trochanter, giv. adductor brevis & pectineus
16. Flat elevated surface of linea aspera.-
17. Triangular popliteal space.-
18. Intercondyloid or crucial notch.-
19. Medullary foramen.

THE FEMUR.

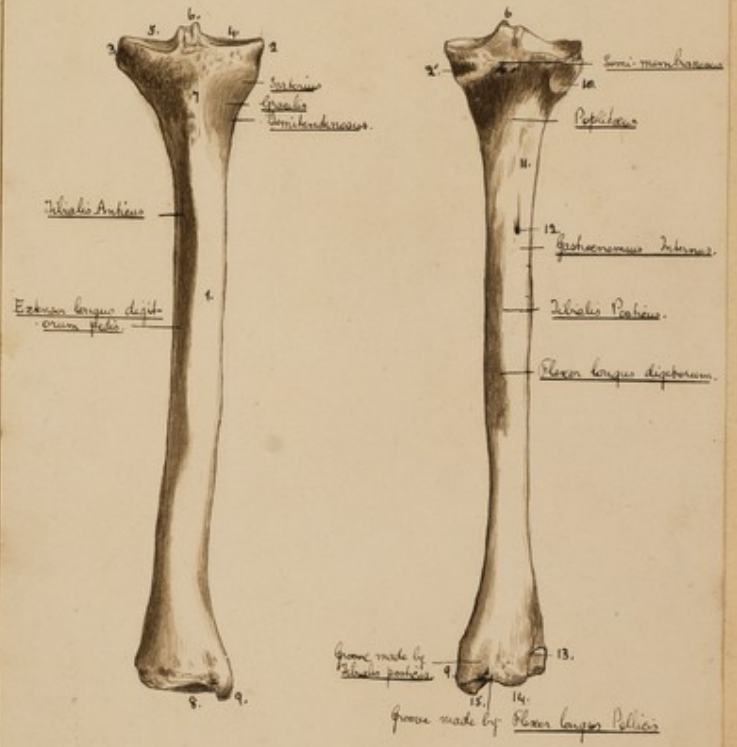

Obturator Externus
Glutæus Minimus
Crureus
Vastus Internus
Pectineus
Vastus Internus
Vastus Externus
Biceps
Ext.ⁿ Gastrocnemius
Popliteus

THE FEMUR.

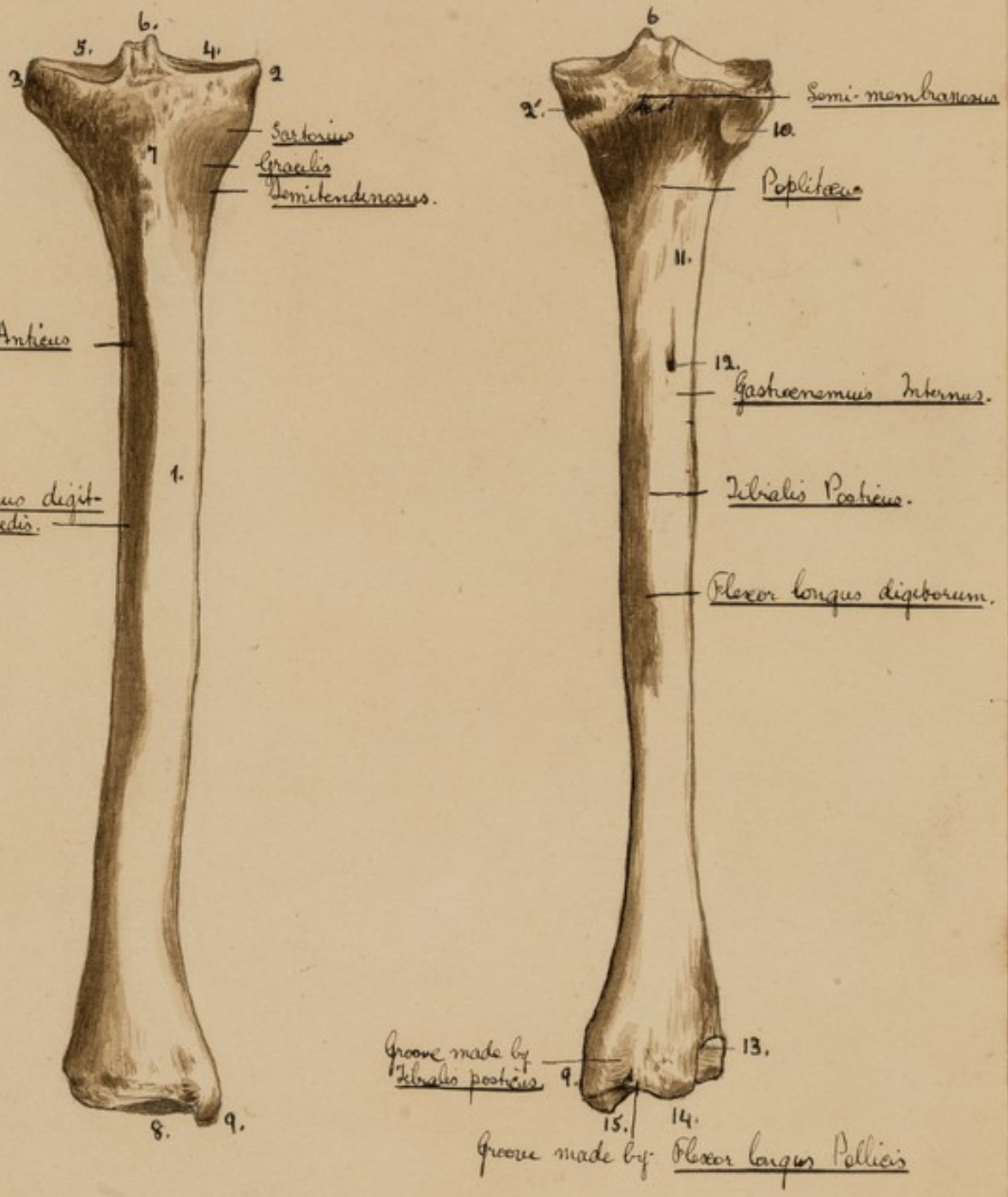

Obturator Externus
Glutæus Minimus
4.
2
3.
Crureus
6.
5. Vastus Internus
1.
10.
12.
9.
Popliteus
13.
8.
11.
7.


Pyriformis (2) Obturators, (2) Gemelli
2'.
4.
3'.
Quadratus Femoris
Glutæus Medius.
Vastus Externus.
Recti
5
Glutæus Maximus.
Biceps { " Longus / Adductor Brevis / " Magnus
14.
15.
Biceps (short head)
Pectineus
11.
Vastus Internus
Vastus Externus.
16.
17.
Biceps.
Ext.al Gastrocnemius
9.
10.
Gastrocnemius
Popliteus
13.
7
18.
8

THE TIBIA

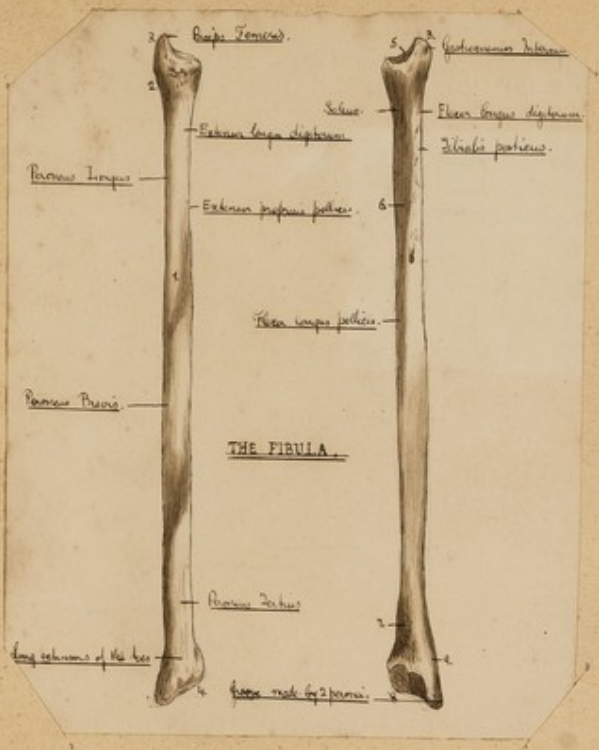

Semi-membranosus
Popliteus
Gastrocnemius Internus.
Tibialis Posticus.
Flexor longus digitorum.
Tibialis Anticus
Extensor longus digitorum pedis
Groove made by Tibialis posticus
Groove made by Flexor longus Pollicis

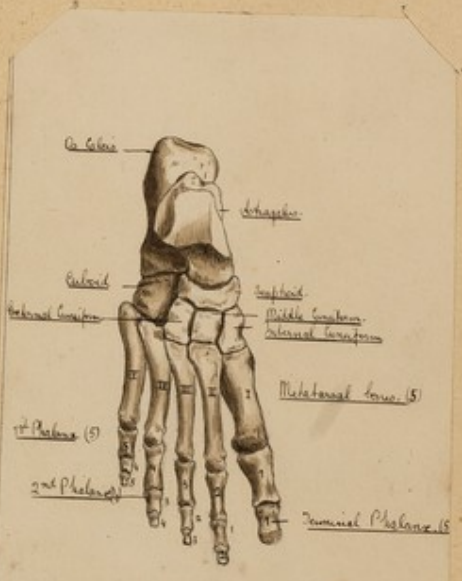
THE TIBIA

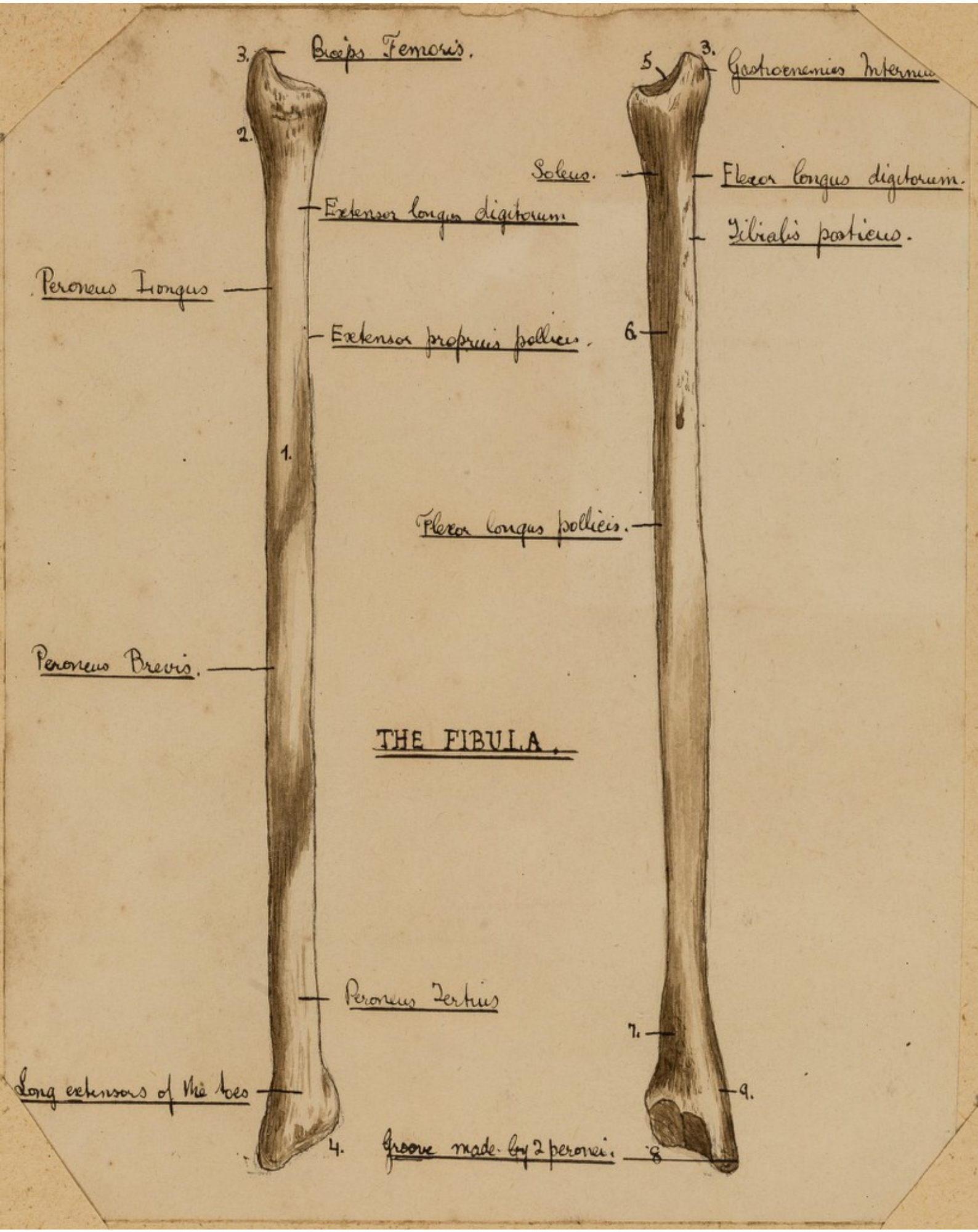

6.
5.
4.
3
2
7
Sartorius
Gracilis
Semitendinosus.
Tibialis Anticus
1.
Extensor longus digitorum pedis.
8.
9.
6
Semi-membranosus
2.
10.
Popliteus
11.
12.
Gastrocnemius Internus.
Tibialis Posticus.
Flexor longus digitorum.
Groove made by Tibialis posticus
9.
13.
15.
14.
Groove made by Flexor longus Pollicis

Male & female pelves contrasted with each other.

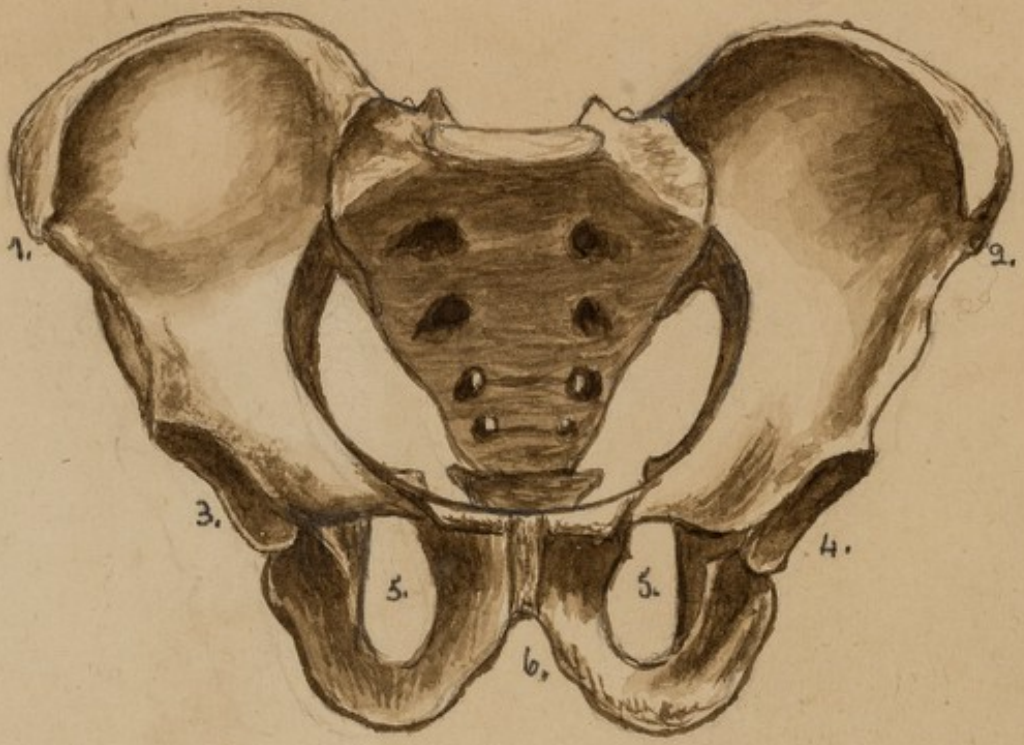
1 & 2. Anterior extremities of the crest of the ilium in front of widest transverse diameter of false pelvis.—
3 & 4. Acetabula.—
5. Thyroid or obturator foramen.—
6. Subpubic angle or arch.—


Peroneus Tertius
Peroneus Brevis
THE FIBULA.

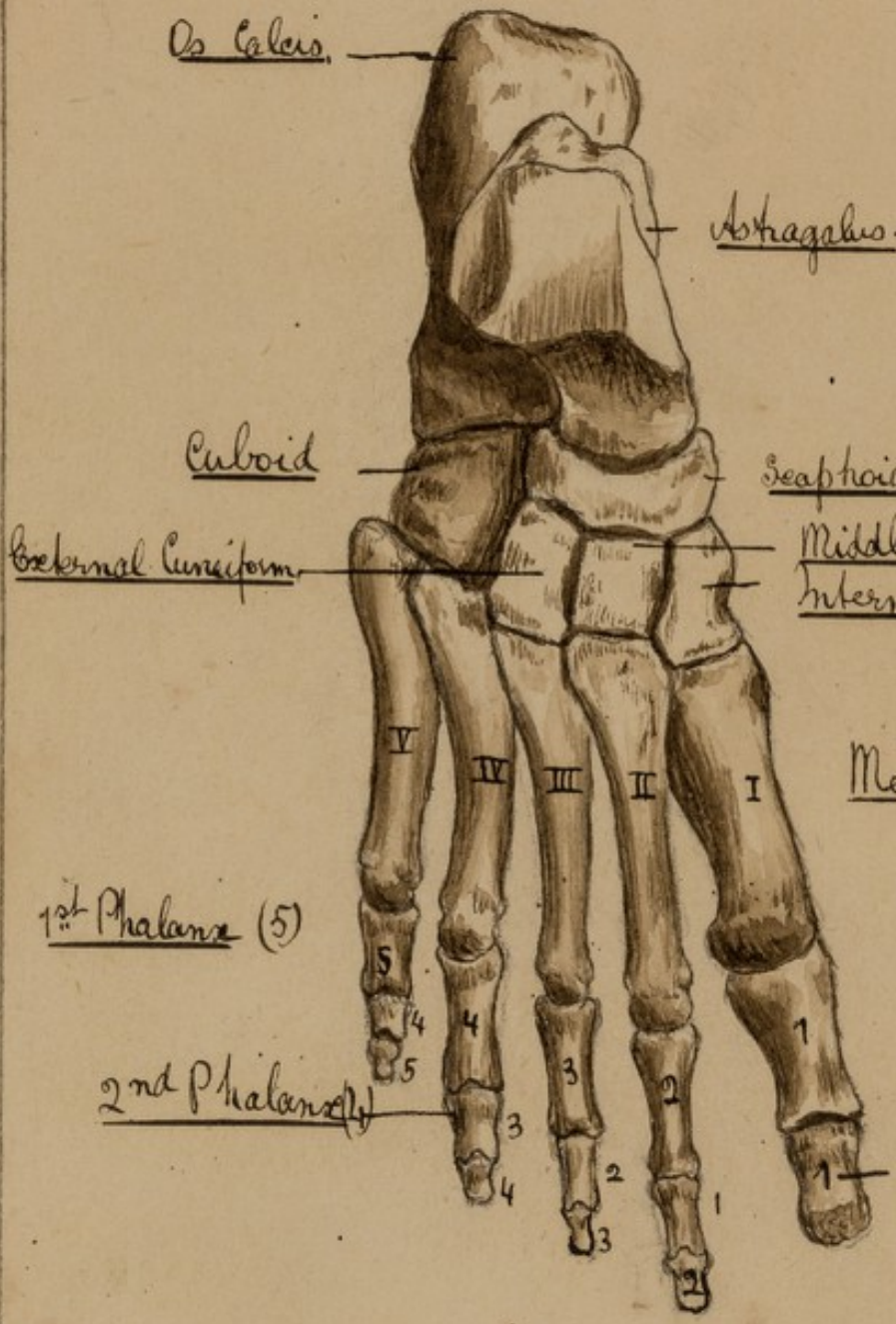

Os Calcis
Astragalus
Cuboid
Scaphoid
Middle Cuneiform
Internal Cuneiform
Metatarsal bones (5)
1st Phalanx (5)
2nd Phalanx (5)
Terminal Phalanx (5)
RIGHT FOOT


3.
Biceps Femoris.
2.
Extensor longus digitorum.
Peroneus Longus
Extensor proprius pollicis.
1.
Peroneus Brevis.
Peroneus Tertius
Long extensors of the toes
4.
5
3.
Gastrocnemius Internus
Soleus.
Flexor longus digitorum.
Tibialis posticus.
6.
Flexor longus pollicis.
7.
9.
Groove made by 2 peronei.
8

THE FIBULA.

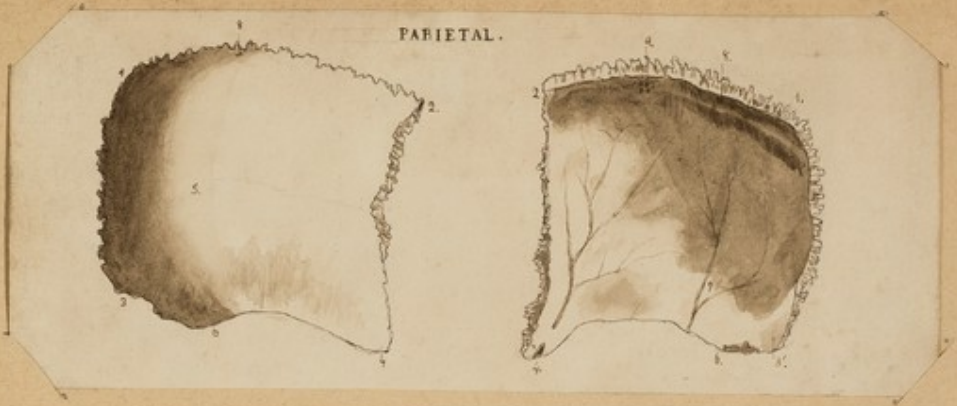

1.
2.
3.
4.
5.
5.
6.


1.
2.
3.
4.
5.
5.
6

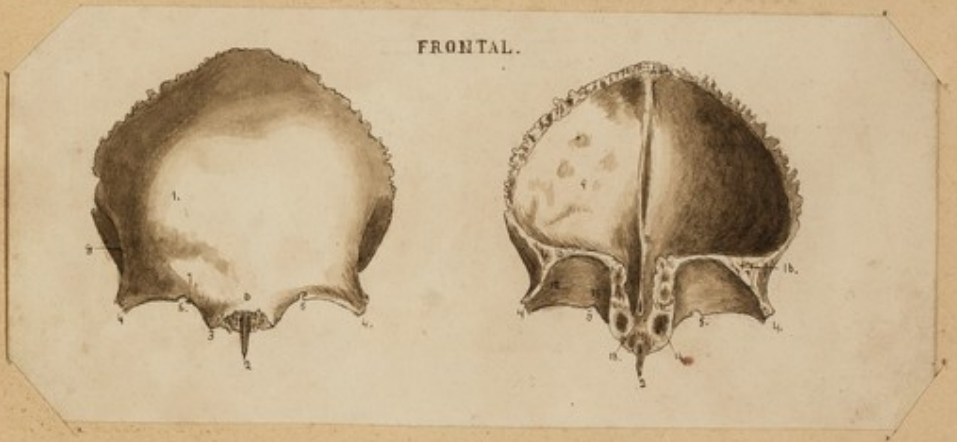

Os Calcis.
Astragalus.
Cuboid
Scaphoid.
External Cuneiform.
Middle Cuneiform.
Internal Cuneiform
V
IV
III
II
I
Metatarsal bones. (5)
1st Phalanx (5)
2nd Phalanx (4)
Terminal Phalanx. (5

RIGHT FOOT

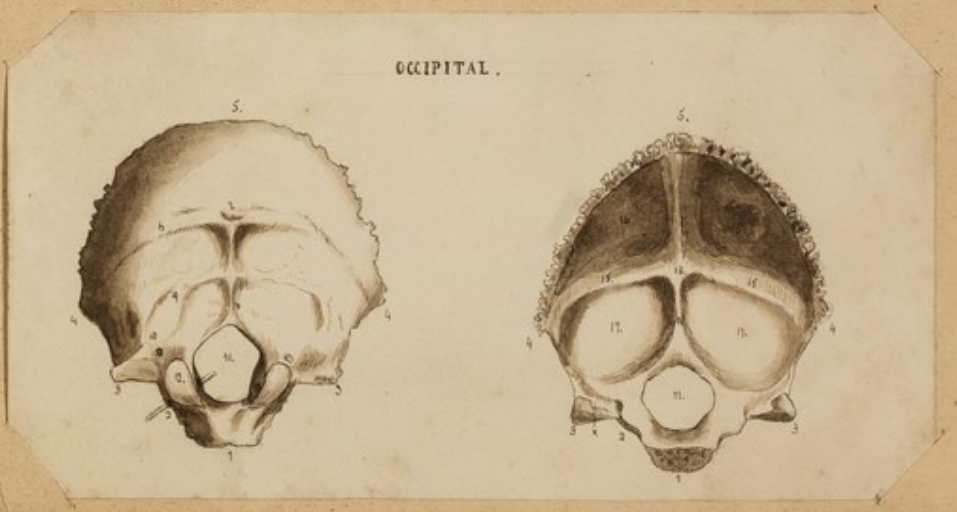
PARIETAL.



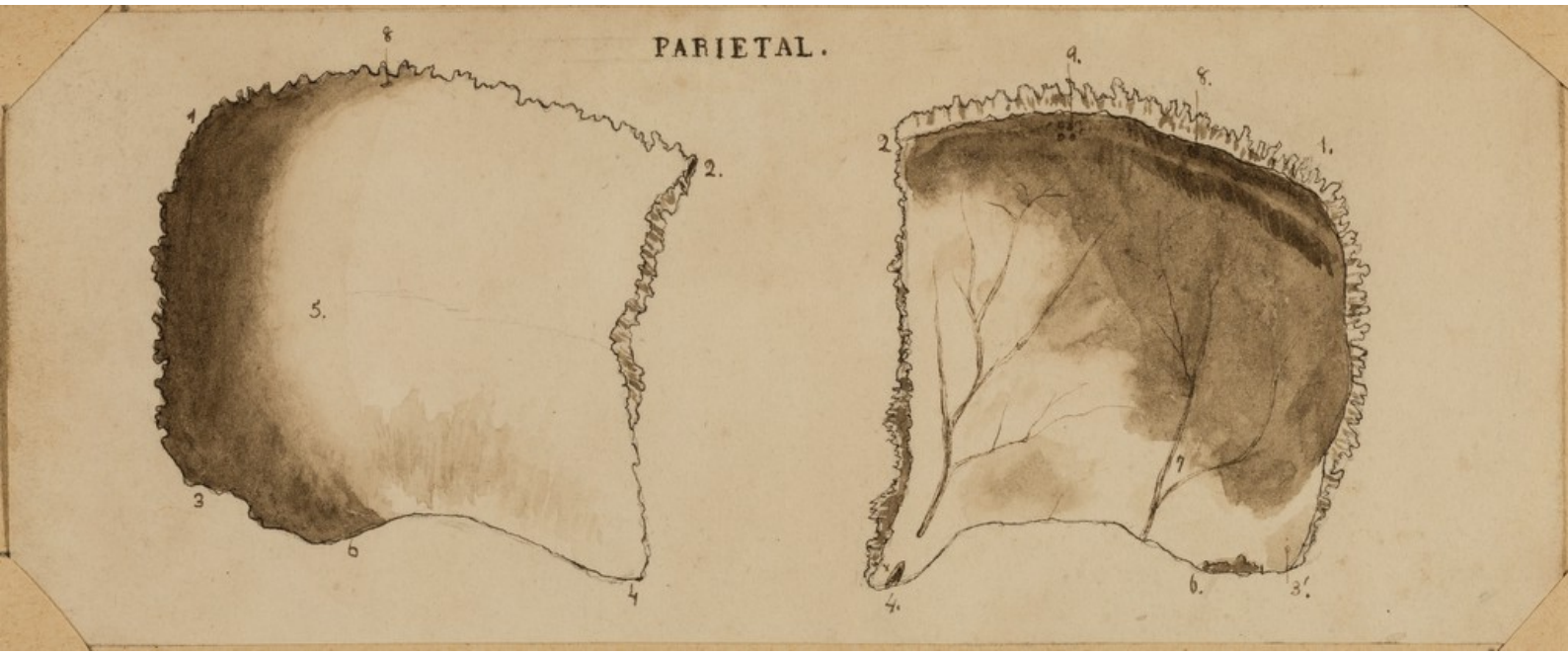
FRONTAL.



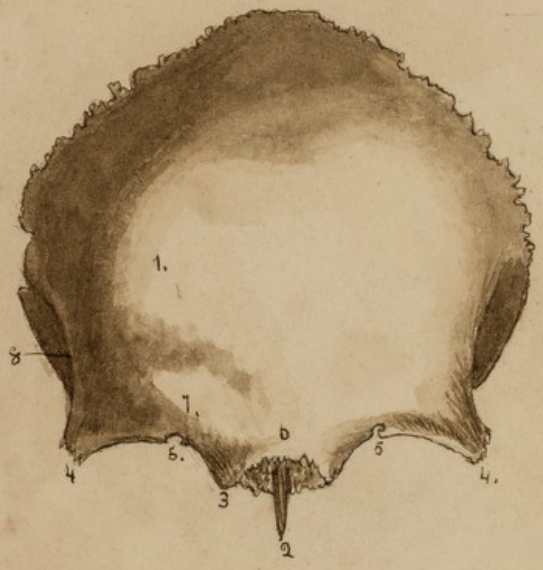
OCCIPITAL.

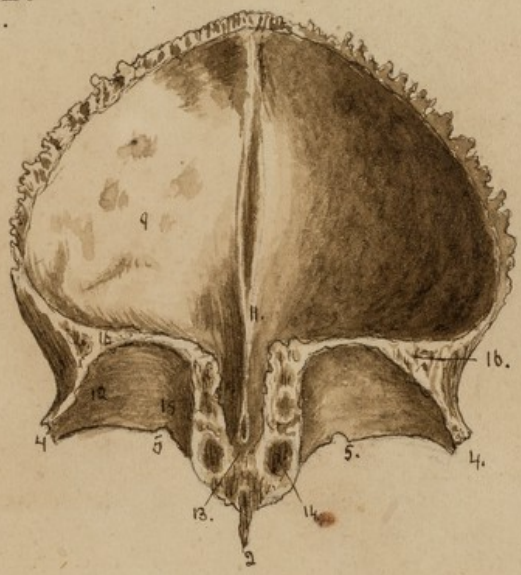


PARIETAL.

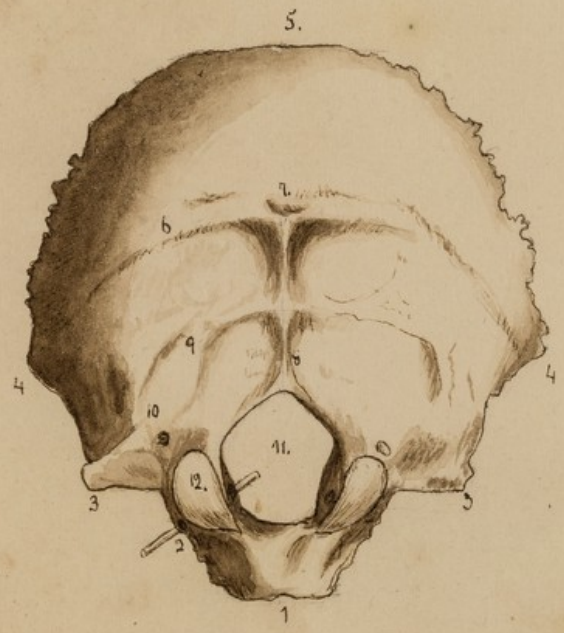


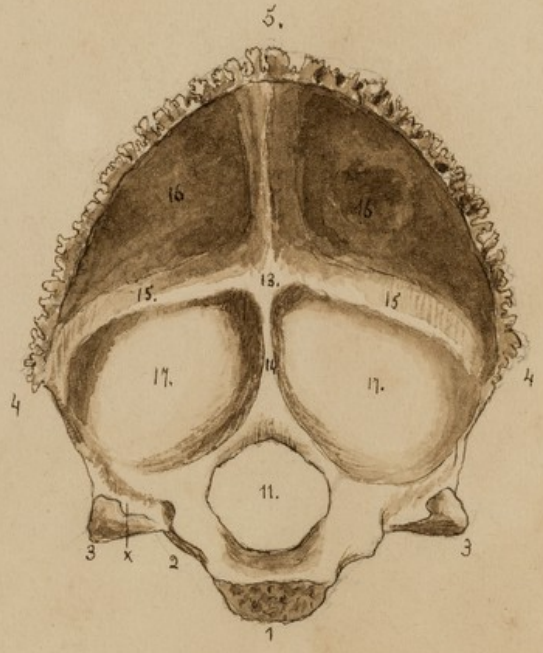
FRONTAL.





OCCIPITAL.

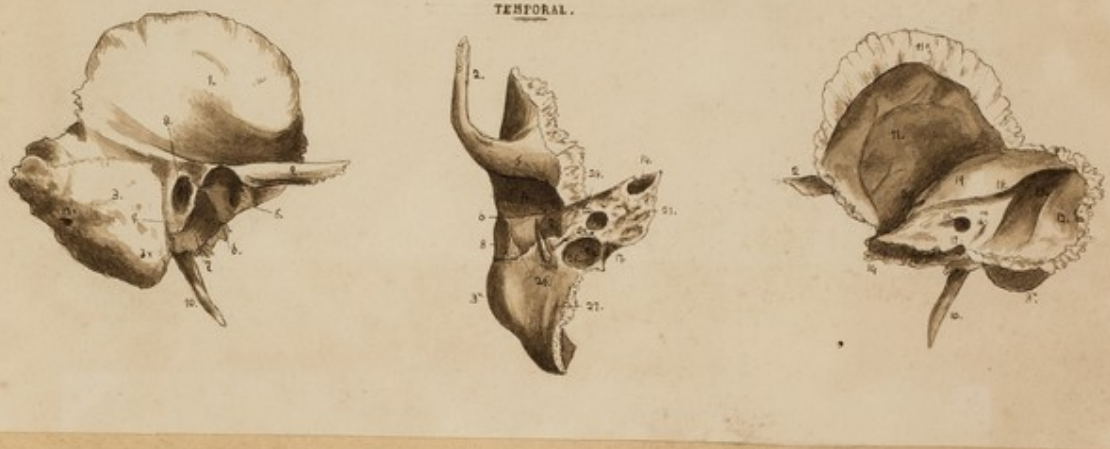




The Sphenoid

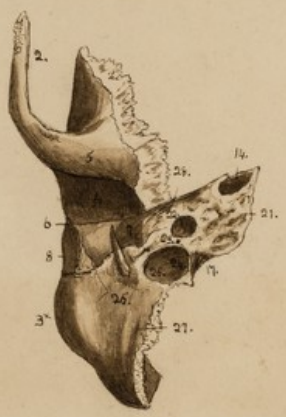
1. Basilar surface seen from occipital.
2. Dorsum sellæ. 3-2 Sella turcica.
3. Olivary process or eminence. in front of 3 a transverse groove of optic commissure.
4. Sigmoid groove of internal carotid artery
5. Lesser wings; & anterior clinoid process
6. Ethmoid spine
7. Cerebral surface of great wings
8. Upper angle of great wings, orbt. or parietal.
9. Spinous process. 10 & 11. Ext. & Int. pterygoid.
11. Opposite lamellar process & groove for tensor palati
12. Optic foramen
13. Sphenoidal fissure or foramen lacerum orbitale.
14. Foramen rotundum. 15. Foramen ovale.
16. Foramen spinosum.
17. Hole posterior opening of Pterygoid or Vidian foramen
18. External or temporal surface of great wing
19. Its orbital surface
20. Sphenoidal turbinated or spongy bone. & above it opening into sphenoidal sinus.
21. Sphenoidal spine.
22. The rostrum & above it the inverted lamina which fits into the edge of the vomer.

TEMPORAL.



SPHENOID.

TEMPORAL.



SPHENOID.

A Number of Blank Pages Follow, which have not been Photographed.